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Toxicity testing with the willow tree transpiration test - 15 years of results

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ABSTRACT BOOK

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Environmental contaminants from land to sea: continuities and interface in environmental toxicology and chemistry

Nantes 2016
SETAC Europe

ABSTRACT BOOK
SETAC Europe 26th Annual Meeting

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This book composes the abstracts of the presentations for the platform and poster sessions of the 26th Annual Meeting of the Society of Environmental Toxicology and Chemistry (SETAC), conducted at La Cité Nantes Congress Center in Nantes, France, from 22 - 26 May 2016. The abstracts are reproduced as accepted by the Scientific Committee of the meeting and appear in order of abstract code, in alphabetical order per presentation type. The poster spotlight abstracts are included in the list of poster abstracts. The presenting author of each abstract is underlined.

Nantes 2016
SETAC Europe

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SOCIETY OF ENVIRONMENTAL TOXICOLOGY AND CHEMISTRY

In the 1970s, no forum existed for interdisciplinary communication among environmental scientists, biologists, chemists, toxicologists as well as managers and engineers others interested in environmental issues. The Society of Environmental Toxicology and Chemistry (SETAC) was founded in North America in 1979 to fill the void. Based on the dynamic growth in the Society's membership, meeting attendance and publications, the forum was clearly needed. SETAC has two administrative offices, in Pensacola, Florida, USA, established in 1990, and in Brussels, Belgium, established in 2003.
A unique strength of SETAC is its commitment to balance the scientific interests of government, academia and business. The Society by-laws mandate equal representation from these three sectors for officers, World Council, Geographic Unit Boards of Directors and Councils, and Committee members and governance of activities. The proportion of members from each of the three sectors has remained nearly equal over the years.
SETAC publishes two globally esteemed scientific journals and convenes annual meetings around the world, showcasing cutting-edge science in poster and platform presentations. Because of its multidisciplinary approach, the scope of the science of SETAC is broader in concept and application than that of many other societies.
The Society is concerned about global environmental issues. Its members are committed to Environmental Quality through Science®, to timely and effective communication of research, and to interactions among professionals so that enhanced knowledge and increased personal exchanges occur. SETAC's growth has been marked the establishment of geographic units around the world: SETAC Europe in 1989, SETAC Asia/Pacific in 1997, SETAC Latin America in 1999 and SETAC Africa in 2012. As evidence of international acceptance of the SETAC model and of the great interest at the local level, regional chapters of the geographic units are being considered for a number of countries.

Publications
Environmental Toxicology and Chemistry, an internationally acclaimed scientific journal, has grown from a quarterly publication of fewer than 400 pages annually in 1980 to a monthly publication of nearly 3,000 pages annually.
Integrated Environmental Assessment and Management, launched in 2005 to bridge the gap between scientific research and its application in environmental decision-making, regulation and management, has become a well-respected quarterly publication of 700 pages annually.
SETAC Books total more than 100, encompassing workshop results and other scientific studies.

Keynote Abstracts

K1
The Plastic Tide
Thomas Maes, Cefas, UK
BIO: Thomas Maes is a recognised science leader with nearly 10 years' experience in many aspects of coastal and ocean monitoring, ranging from chemical pollutants and related biological effects to marine litter and other emerging compounds. He has good interpersonal skills and is an effective communicator in different languages. Thomas is Cefas` International and National Monitoring Programme Coordinator who joined Cefas in 2008 after an international career as environmental manager for large marine development projects. He coordinates and develops Cefas` international monitoring programmes and provides advice to UK Government issues related to human activities such as contaminant and other emerging pressures. He is involved in the assessment of data and adaptation of monitoring programmes for further implementation of the MSFD. Thomas is currently also chairing the OSPAR monitoring and assessment group (MIME) and acting as the UK expert on Marine Litter in the European task groups. He manages large, multi-disciplinary research projects and monitoring programmes including leadership of several EU-funded programmes in relation to contaminants and marine litter (e.g.: MICRO, MARLISCO,...).

K2
Climate Change and Environmental Contamination by Chemicals: Do They Affect Ecological and Human Health?
Arja Rautio, Thule Institute, University of Oulu, Finland
New emerging contaminants, persistent organic pollutants (POPs) and toxic metals are transported to the Arctic from regions at lower latitudes. During the last twenty years the Arctic Monitoring and Assessment Programme (AMAP) has followed the levels of POPs and metals in environment, wild-life and human populations, especially pregnant women. Most POPs and metals are declining in many Arctic regions, but there are some areas and populations where levels remain high. Elevated concentrations of toxic metals (usually mercury) and environmental contaminants (dioxins and PCBs) have been measured in Greenland and Canada from seafood, like marine mammals and fish, and dietary advice is needed for users of traditional and local foods. Interactions between climate change and contaminant transport have the potential to change human exposure in the Arctic significantly. It is not possible to estimate the likelihood and magnitude of the health impacts and the changes in exposure.
The incidence of many Western type of diseases, like diabetes, heart diseases and cancers, has increased globally, and high increase in numbers have been found among people living in the Arctic. It has been assumed that environmental pollutants may have a contributing effect especially in some heavily exposed Indigenous populations. Epidemiological studies have shown the connections between contaminant exposure (especially mercury) and changes in foetal growth and child development and cardiovascular effects. However, investigations on the precise impact of environmental pollutants on human health are difficult to study, because there are many factors, which affect simultaneously and varying degrees. These include genetic background, age, sex, diseases, exposure history and environment. Environmental factors include mixtures of contaminants and other chemical compounds to which individuals are commonly exposed. Knowledge about the effects of mixtures is mainly missing and difficult to study at population level.
Exposure during the foetal period is in special attention in the epidemiological and mechanistic (in vitro) studies, because foetal stage is the most vulnerable during human life. During the last decade there have been several research projects under the framework of the EU, which have focused on potential toxicity at low exposure levels of environmental contaminants to child development (projects like ENRICO, OBELIX, CLEAR, INUENDO, ArcRisk, PHIME). One goal of this research has been to collect all the existing evidence about the associations between environmental contaminants and measured health outcomes. The evaluation of potential human health risks and their magnitude is needed.
Changes in climate, environmental and land use together with socioeconomic factors have impact on the contaminant exposure for wild-life and humans.

BIO: Arja Rautio, research professor, MD, PhD, Eurotox ERT, has been working in the field of circumpolar health since 2006 in the Thule Institute and Faculty of Medicine, University of Oulu, Finland. She is leading and participating in several national and international research projects, which focus on climate change, environmental health, risk assessment, and indigenous health and wellbeing. She is a chair of the University of the Arctic Thematic Network of Health and Wellbeing in the Arctic (www.uarctic.org), and is leading the international Master's and Doctoral programs on Circumpolar Health and Wellbeing. Dr Rautio is working as a national key expert in the Human Health Expert groups of the Arctic Monitoring and Assessment Programme (www.amap.no) and Sustainable Development Working Group (www.sdwg.org). She has been nominated as a national member to the International Arctic Science Committee - Social and Human Sciences Group (http://iasc.arcticportal.org). She is a chair of the Nordic Society of Circumpolar Health.

K3
Environmental Assessment of Cosmetics and Personal Care Products: Challenges and opportunities
Marc Leonard, L'Oréal Advanced Research, France
Despite a frequent image of futility, cosmetic and personal care products provide strong societal benefits. For centuries, their use has been motivated worldwide by the continuing need for hygiene, beauty and self-esteem.
Nowadays, cosmetics and personal care products (CPCP) are most frequently used in the bathroom. After rinsing they flow down the drain where they mix with wastewaters. In industrialized countries they are directed to sewage treatment plants, but in many developing countries wastewaters may directly be discharged into rivers or the sea shore. In addition, certain products such as sunscreens may be directly released by swimmers in lakes, rivers or the ocean.
The exposure of the environment to cosmetic and personal care products is relatively low compared to contaminants from other industrial sectors but nonetheless, the environmental risk assessment of their constituents is done in a similar manner. Assessing their potential environmental impact faces significant methodology challenges because of their extremely diverse composition, from single ingredients to heterogeneous complex mixtures such as natural extracts and essential oils.
In parallel, there is a worldwide trend to move away from animal testing for the human and environmental safety assessment of cosmetics and personal care products. In this regard, fish which are key aquatic models in environmental risk assessment, fall into the scope of several international regulations for the protection of animals used for scientific purposes. As a consequence, replacing animal testing for the safety assessment of cosmetic and personal care products faces additional challenges when addressing environmental issues such as fish long term toxicity, environmental endocrine modulation and bioaccumulation, where fish BCF data are still required for regulatory PBT/vPvB classification.
Cosmetic companies share with authorities the desire to inform the public which products have the best environmental profile. Thus, several projects are under study at national (e.g. French) and international (e.g. EU) levels for consumer products environmental footprint labelling, including cosmetics and personal care products. They plan to assess the impact of products on aquatic ecosystems with the USEtox model, developed for Life Cycle Assessment (LCA). But some deviating results are obtained when using other methodologies such as the Critical Dilution Volume (CDV) calculation, which is required to award the European Ecolabel for cosmetics products. Concordant and relevant methodologies are needed and progresses are being made in this direction.
Nonetheless, this challenging context provides opportunities to develop alternative methodologies to anticipate potential short and long term adverse environmental effects of cosmetic and personal care products. Benefits are even expected from the field of human toxicology screening, where aquatic models such as the fish embryo are gaining much interest.
The purpose of this presentation will be to present the trends and advances in these fields.

BIO: Marc Leonard is presently head of L'Oreal Research & Innovation, Environmental Research Department. He obtained his doctorate in Veterinary Medicine from the Veterinary School of Maisons-Alfort (France), with a specialization in environmental toxicology from the Centre des sciences de l'Environnement - Metz University (France). The main activities of his department cover two domains : (1) Environmental Assessment of raw materials and cosmetic preparations with applied research aimed at developing cost-efficient assessment tests as potential alternatives to the corresponding OECD guidelines. Domains cover acute and chronic aquatic toxicity (soft and marine waters), bioaccumulation and biodegradation assessment. (2) Invertebrates and Fish Embryo models as potential alternative methods in Human Toxicology and Pharmacology.
He has been co-chairman of the HESI (Health & Environmental Sciences Institute) Committee on the Emergence of Animal Alternative Needs in Environmental Risk Assessment. He was member of the OECD Fish Embryo Toxicity Expert Group, and OECD Expert Group on Invertebrates Reproduction. He has hosted and co-organized several international workshops on the Fish embryo model with international partners such as HESI and UFZ (Helmotz Center) and contributed to publications in these fields.

Platform Abstracts

Contaminants of Emerging Concern in the Environment and their Management (I)

1
Solutions for present and future emerging pollutants in land and water resources management
W. Brack, Helmholtz Centre for Environmental Research UFZ / EffectDirected Analysis; R. Altenburger, UFC Centre for Environmental Research / Bioanalytical Ecotoxicology; D. Bunke, Öko-Institut e.V. / Sustainable Products Material Flows Division; W. Busch, Helmholtz centre for environmental research - UFZ / Bioanalytical Ecotoxicology; G. Engelen, Flemish Institute for Technological Research VITO; B.I. Escher, Helmholtz Centre for Environmental Research UFZ / Cell Toxicology; B. Gawlik, European Commissions Joint Research Centre JRC; M. Krauss, Helmholtz centre for environmental research - UFZ / Effect Directed Analysis; C. Lindim, Stockholm University; I. Liska, International Commission for the Protection of the Danube River ICPDR; J. Munthe, IVL Swedish Environmental Research Institute Ltd; P.A. Neale, Griffith University / Smart Water Research Centre; L. Posthuma, RIVM / Centre for Sustainability Environment and Health; T. Schulze, Helmholtz centre for environmental research - UFZ / EffectDirected Analysis; F. Sleeuwaert, Flemish Institute for Technological Research VITO; J. Slobodnik, Environmental Institute; J. van Gils, DELTARES; A. van Wezel, KWR Watercycle Research Institute / Chemical Water Quality and Health
Despite significant efforts the good ecological status required by European Water Framework Directive (WFD) could not be achieved in the majority of European surface waters. Emerging pollutants such as pharmaceuticals, biocides, personal care products and many others are hypothesised to play a key role for water quality. The 5-year Collaborative Project SOLUTIONS addresses complex mixtures of potentially toxic compounds in the water cycle including legacy, presently used and future chemicals with monitoring-, modelling- and scenario-based approaches focusing on the assessment and prediction of the impact of these mixtures, on the identification of drivers of mixture toxicity focusing on deriving candidate River Basin Specific Pollutants (RBSPs) and on the identification of priority mixtures. For the identification of priority mixtures with respect to specific targets different criteria are used to establish chemical fingerprints based on common sources, modes of action, fate etc.. The impact of mixtures in the environment is addressed by the development and demonstration of a set of effect-based tools following the philosophy of adverse outcome pathways from key events on a molecular level via cellular and organism responses up to the community. These tools are evaluated and demonstrated in large scale case studies such as Rivers Danube, Rhine and Ebro. Results from the River Danube indicate low to moderate effects on a large range of endpoints, most of them explained only to a minor extent by target analytes. Prospective assessment using an integrated system of models and databases provides extensive predictions of concentrations and risks in the Danube river. First evaluations for individual chemicals such as PFOS and PFOA indicate good agreement between monitoring and modelling. Both approaches together are used to propose RBSPs.

2
Lagrangian tracer aided sampling approach in assessing the fate of wastewater-related contaminants in rivers
G. Guillet, Tübingen University / Applied Geoscience; J. Knapp, Eberhard Karls Universität Tübingen / Hydrogeology Center for Applied Geosciences; M. Schwientek, Eberhard Karls Universität Tübingen / Hydrogeochemistry Center for Applied Geosciences; S. Merel, Eberhard Karls Universität Tübingen / Environmental Analytical Chemistry Center for Applied Geosciences; O. Cirpka, Eberhard Karls Universität Tübingen / Hydrogeology Center for Applied Geosciences; C. Zwiener, Eberhard Karls Universität Tübingen / Environmental Analytical Chemistry Center for Applied Geosciences; P. Grathwohl, Eberhard Karls Universität Tübingen / Hydrogeochemistry Center for Applied Geosciences
Emerging contaminants are constantly released by Wastewater Treatment Plants (WWTPs) into surface waters. The persistence of these compounds influences how far they will spread within the river network, raising concern on the extent of their environmental impact and the issue for drinking water exploitations. To study the fate of wastewater-related pollutants in rivers, a common approach is using a Lagrangian sampling scheme. It consists in sampling the same parcel of water repeatedly as it flows along a selected river stretch. Where this approach allows monitoring concentration changes according to residence time, it may include uncertainties that can be misinterpreted as removal – diurnal pattern of WWTPs' effluent coupled with inaccurate travel time estimation and dispersion effects. A new sampling approach combining Lagrangian sampling with an artificial tracer was applied to study the fate of wastewater-related compounds in an impacted river segment. The tracer fluorescein was injected upstream of a 1,7 km long stretch of the Steinlach River, southwest Germany, downstream of a WWTP effluent. The segment was controlled by four measuring stations during a day and a night experiment. Normalizing the compounds concentrations to the tracer isn't possible in the context of wastewater-related compounds, constantly released unlike the tracer. Instead, breakthrough curves were deconvoluted to identify transfer functions between each measuring stations. Transfer functions were then applied to a concentration time series recorded by six 1h composite samples at the first sampling site. The obtained time series at each control plane were then compared to the concentration measured from a grab sample. Different behaviours from conservative to very reactive could be observed for the 43 investigated compounds. Compounds like the pharmaceuticals diclofenac or tramadol showed a strict photosensitive character. Other compounds were attenuated at a similar strength during day and night (atenolol, valsartan) and others displayed both behaviours – with a higher elimination during day time (bisoprolol, DEET or TAED). Part of the compounds list was investigated in a previous experiment also performed in the Steinlach but at only two control planes in July 2013. Similar behaviour were found with however a better attenuation in the last experiment. Finally, unlike 2013 sampling, 2015 experiment could supply decay profiles, particularly interesting for compounds like DEET.

3
Abiotic and biotic fate of lamotrigine N2-glucuronide in wastewaters and surface waters
B. Zonja, S. Perez, IDAEA-CSIC / Environmental Chemistry; D. Barcelo, IIQAB-CSIC / Dept Environmental Chemistry
Lamotrigine, an anticonvulsant, is a xenobiotic which is extensively and predominantly transformed by phase II metabolism to its N2-glucuronide before it is excreted from the human body. Both parent and metabolite have been detected in the wastewater system, surface and groundwater. In this work, we tested a suspect-screening approach in order to detect lamotrigine and its related compounds like other human metabolites, impurities and phototransformation products in the aquatic system. Environmental samples that were preconcentrated using generic solid-phase extraction and analysed by liquid chromatography - high resolution mass spectrometry method suggested lamotrigine undergoes unusual transformations. However, biodegradation reactors amended with mixed liquor at neutral pH showed that lamotrigine is resistant to biodegradation with only about 5 % elimination after 6 days. When its human metabolite (N2-glucuronide) was degraded following the same experimental setup, it was discovered that the metabolite is in fact the source of the transformation products (TPs) detected. In batch experiments, N2-glucuronide was transformed to three TPs as a result of i) deconjugation, ii) oxidation of the glucuronic acid and iii) amidine hydrolysis in combination with deconjugation. All these compounds were detected in wastewater treatment plant (WWTP) influent and effluent, and surface water samples analysed. Additionally, in the WWTPs, these TPs helped explain the mass balance of the N2-glucuronide. Apart from the biotic reactions, the abiotic stability of the glucuronide in the pH range 4 - 9 was tested in order to characterise the possible formation of amidine and guanidine hydrolysis TPs. This was performed in various environmental matrices. Other N2-supsituted lamotrigine derivatives were tested as well in order to test the influence of N2-supsitution on reaction kinetics. Results showed that N2-glucuronide was transforming via amidine (primarily) and guanidine hydrolysis under neutral-basic pH and the transformation rate was exponentially increased with the increase of pH. Moreover, this reaction was shown to depend on imine tautomer equilibrium and imine tautomer formation depended directly on the N2-supsitutent. In the end, the presented work intents to give a new insight into the behaviour of glucuronides of pharmaceuticals, and suggest that they can also be sources of environmentally relevant but yet undiscovered TPs.

4
Micropollutans - how to cope with the residues?
A. Zenker, University of Appl. Sc. Northwestern Switzerland / Institute of Ecopreneurship; T. Wintgens, University of Applied Sciences Northwestern Switzerland (FHNW), School of Life Sciences, Institute of Ecopreneurship; J. Loewenberg, CSD Ingenieure AG; J. Svojitka, R. Hochstrat, University of Applied Sciences Northwestern Switzerland (FHNW), School of Life Sciences, Institute of Ecopreneurship; S. Schmidt, University of Applied Sciences Rapperswil, Institute of Environmental and Process Engineering; M. Burkhardt, UMTEC, HSR Hochschule für Technik Rapperswil
Most recently Switzerland as one of first countries worldwide has changed its Water Protection Ordinance demanding about 100 wastewater treatment plants (WWTP) to upgrade with an additional treatment step. The additional step should help reducing 80% of micropollutants' load of the influent over the whole sewage treatment process. Micropollutants are present at low concentrations in aquatic environment ranging from ng/L - μg/L, but their negative effects on biota have already been demonstrated. Ozonation and the adsorption of micropollutants by powdered activated carbon (PAC) are currently the most promising techniques to eliminate micropollutants in WWTPs. An overview of different techniques to eliminate micropollutants in waste water, stormwater and drinking water will be presented. Different adsorber materials like PAC (SAE Super, Sorbopor MV 125, Carbopal AP, Pulsorb WP 260), granular activated carbon (GAC) and resin based adsorbers were tested in combination with flotation, deep bed filtration and

ultrafiltration to reduce micropollutants. These settings were tested under laboratory conditions, in pilot scale and subsequently upscaled in the treatment plants themselves. To monitor the removal lead substances from 48h composite samples including pharmaceuticals such as carbamazepine, diclofenac, sulfamethoxazole, the herbicide mecoprop and the corrosion inhibitor benzotriazole were determined. Measurements were carried out on a high performance liquid chromatography coupled to triple quadrupole mass spectrometer. It was shown that the combination of PAC and filtration or PAC and flotation are suitable technologies to remove nearly 80% of the micropollutants' load of urban and industrial wastewater. During the operation duration of several months the removal was robust and less prone to faults.

5
Do pharmaceuticals bioaccumulate in marine molluscs and fishes from a coastal lagoon?
V. León, R. Moreno-González, Instituto Español de Oceanografia / Centro Oceanográfico de Murcia; S. Rodriguez-Mozaz, Institute for Water Research (ICRA) / Water Quality; B. Huerta Buitrago, Catalan Institute for Water Research (ICRA) / Institute of Environment Health and Societies; D. Barceló, IDAEA-CSIC / Department of Environmental Chemistry
The pressure and impact of organic pollutants are greater in coastal areas than in the rest of the marine environment, since these are where many human activities are concentrated and direct and indirect discharges occur. In the marine environment, pharmaceuticals have predominantly been found in seawater, but less so in sediment. However, field data of the bioaccumulation of pharmaceuticals in marine organisms exposed to environmental conditions is rather sparse and concentrations of pharmaceuticals in marine fish have only been determined in a small number of areas. The aims of this study were to determine the concentration of pharmaceuticals in cockle, noble pen shell, sea snail, golden grey mullet and black goby in spring and autumn; and to assess the bioaccumulation in cages clams in 4 sites with different exposition grade to El Albujón watercourse dischargue. A method proposed for the determination of 20 multi-class pharmaceuticals in fish tissues (was adapted for grey golden mullet muscle and liver and for the following molluscs: clam, noble pen shell, and sea snail. Eighteen out of the 20 compounds analyzed were found at low ng g^{-1} in these species throughout the lagoon. Hydrochlorothiazide and carbamazepine were detected in all species considered. The bioaccumulation of pharmaceuticals was heterogeneous in the lagoon, with a higher number of pharmaceuticals being detected in fish (19) than in wild molluscs (10), particularly in golden grey mullet muscle (16). The bioaccumulation of pharmaceuticals was lower in sea snail than in bivalves, and in black goby than in golden grey mullet. Psychiatric drugs preferentially bioaccumulated in fish muscle, while citalopram did so in molluscs. Carbamazepine and hydrochlorothiazide were detected in all species in this study. The higher detection frequency and concentrations found in golden grey mullet (muscle) suggested that mugilids could be used as an indicator of contamination by pharmaceuticals in coastal areas. *Acknowledgement* - This work was supported by the Seneca Foundation (Region of Murcia, Spain) through the 'BIOMARO' project (15398/PI/10), the Spanish Inter-Ministerial Science and Technology Commission through the 'IMPACTA' project (CICYT, CTM2013-48194-C3-1-R) and by the European Union through the European Regional Development Fund (ERDF). It was also partly supported by the Generalitat de Catalunya (Consolidated Research Group: Catalan Institute for Water Research 2014 SGR 291).

6
Impacts of compound properties and sediment characteristics on the sorption behavior of pharmaceuticals in aquatic systems
O. Abdullah, University of York / Environment; A. Boxall, University of York / Environment Department
Sorption is akey factorin determining the persistence and attenuation of pharmaceuticals in sediment and will therefore affect the impact that a pharmaceutical has on aquatic organisms. However, our understanding of the relationships between sediment characteristics and pharmaceutical sorption behavior is lacking as most studies into the sorption behaviour of pharmaceuticals focus on their behaviour in sewage sludge and soil.In this study, the sorption behaviour of five different pharmaceuticals in ten sediments from the UK and Iraq, with different properties, was assessed. Batch sorption studies with the pharmaceuticals were performed and the sorption affinity for all sediments was found to increase in the ordermefenamic acid < cimetidine < atenolol < amitriptyline < diltiazem.An existing predictive model for estimating the sorption of ionisable compounds was evaluatedagainst the experimental data. The model tended to over-predict the sorption of the basic compounds and under-predict the sorption of the acidic compounds. Multiple linear regression analysiswas therefore used to development new models for estimating the sorption (Kd) of the study pharmaceuticals from sediment properties. Sediment and pharmaceutical parameters used in the models were: (a) log Dow (lipophilicity corrected for pH) for amitriptyline, (b) cation exchange capacity for atenolol, (c) clay and organic carbon content for cimetidine,(d) log Dow and exchangeable Ca^{2+} for diltiazem and (e) organic carbon contentfor mefenamic acid. The validity of the proposed regression equations was tested using independent data and gave good results for atenolol. Overall, the results demonstrate how complex the processes driving the sorption of pharmaceuticals in sediments are and the need for generation of further experimental dataand additional model development for estimating sorption of pharmaceuticals.

Microplastics in the environment: Sources, Fate and Effects (I)

7
Micro- and mesoplastic in Atlantic cod (Gadus morhua) from the Norwegian coast
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Microplastics contaminate oceans and affect marine organisms in several ways. This study documents microplastic (< 5mm) and mesoplastics (>5mm) in stomachs from cod (Gadus morhua) one of the most common and economically important marine fish in Norway. 302 fish stomachs were examined from six different locations from the coast of Norway. Ten individual stomachs had items in them identified as synthetic polymers, eight of these were from one location (Bergen). All objects found in the stomachs were Fourier Transform Infrared Spectroscopy (FTIR) scanned and subsequently compared with FTIR libraries to confirm identity of the removed items. In this study all stomachs where plastics were found, were full. Empty stomachs had no presence of plastic.\nThis study provides a record of plastic polymers being identified in the stomachs of cod in three out of six locations. We found the presence of polyethylene terephtalate (PET or PETE) and polyester, polyvinyl chloride (PVC), polypropylene (PP), polystyrene (PS), acrylic and nylon (Other). We identified a hot spot for plastic ingestion in Bergen. Our findings indicate that plastic pieces are more prevalent inside fish with a full stomach content versus those with empty stomachs. Therefore, pieces large enough to be visually identified through a stereo microscope may be flushed out of the stomach region of the GI tract. Further work is needed to establish the sources and potential consequences of microplastics.

8
Fragmentation of plastic items into secondary microplastics under environmental conditions
J. Klasmeier, University of Osnabrueck / Institute of Environmental Systems Research; A. Reuwer, Osnabrück University / Institute of Environmental Systems Research
Marine plastic debris of all size classes contaminates marine waters and coastlines worldwide. Larger plastic items are suspected to be fragmented under external friction exerted by water currents, rocks or sediments. Weathering, abrasion or disruption of plastics may result in an increasing number of secondary plastic debris. Main goal of this research was the systematic investigation of the fragmentation behavior of plastic material under conditions typically experienced in the marine environment. PE bottles (1 L) were filled with 1 kg pre-cleaned sand (beach conditions), and half of the samples were additionally spiked with 300 mL artificial seawater (breakwater conditions). Plastic items of different polymer material (PE, PP, PS, PET) and form (cups, small bottles, bottle caps, thin foils, film strips) were addd and shaken for 30 days in an overhead shaker. After 30 days, intact items and larger plastic fragments were sorted out by hand. Samples were extracted for microplastic particles using a simplified adaptation of a flotation method developed in our lab. Extracted particles were visually inspected under a microscope and sorted into different size classes. Some randomly selected particles from each size class were analyzed with FTIR (Bruker Vertex 70) to identify the polymer material. All investigated items bear visible traces of mechanical abrasion. Film strips and bottle caps did not fragment, but abrasion of material from the surface had obviously occurred. Thin foil sheets were partly perforated and fragmented into a number of mesoplastic and large microplastic particles of elongated shape. The other investigated items were fragmented into more or less smaller particles after 30 days. Microscopic size analysis of the PS cup sample resulted in four mesoplastic fragments, 35 large microplastic particles (1 – 5 mm) and 32 small microplastics (

9
Analysis of microplastic in environmental samples using thermal analytical methodes
U. Braun, E. Duemichen, A. Barthel, BAM- Federal Institute Materials Research and Testing; N. Becker, C. Bannick, UBA Umweltbundesamt
The increasing amount of polymers in practical use with their excellent material properties, such as low weight, easy processing and high stability against various chemical attacks, will also increase the unfavoured presence of plastics in the environment. These plastics and small-degraded plastic particles, called microplastic, are almost ubiquitary in nature. Up to now, no verified data exist about the source, the fate and the amount of various plastics in water, soil, air and biota. The reason for this is that no fast and easy available method exists, which detect the small amount of synthetic polymers beside a large amount of natural

macromolecules in various environmental matrices. Known, microscopic-spectroscopic methods are limited due to very large measurement times, high sophisticated evaluation knowledge or method specific limitations such as sample preparation in infrared analysis or fluorescence of environmental matrix in Raman. Furthermore, no quantitative value can be determined by these methods. Quantitative values, which include the whole range of synthetic polymers, are a prerequisite for administrative regulations. A practicable alternative is the thermal extraction desorption- gas chromatography-mass spectroscopy (TED-GC-MS) [1]. In this method, the advantages of TGA (high samples loadings and an easy cleaning) are combined with the advantages of GC-MS (easy identification of various hydrocarbon products with high certainty). In TGA, a large amount of sample is decomposed. The formed volatile decomposition products can be trapped on the surface of an adsorber material located at the outlet of TGA and collects a representative content of hydrocarbon decomposition. The decomposition products are analysed in a common TDS-GC-MS. The comfortable identification of characteristic decomposition products enables the fast identification of various polymers. Beside the investigation of various spiced environmental samples for the presentation of this new method, we present data of real environmental samples. These are air and water filtrates from various sources as well as samples from composting plants and streets. Samples are measured as received or after treatment with chemical solutions. [1] E. Dümichen, A.-K. Barthel, U. Braun, C. G. Bannick, K. Brand, M. Jekel and R. Senz, *Water Research* **2015**, *85*, 451-457.

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Role of microplastic beads on the uptake of silver in zebrafish

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This study aimed to determine whether the uptake and localization of Ag in zebrafish was affected by the presence of polyethylene microplastic beads (PE MPBs). Zebrafish were exposed to 1 μg Ag L^{-1} (radiolabelled with ^{110m}Ag) for 4 and 24 h in the presence or absence of PE MPBs (10, 100 or 1000 MPBs mL^{-1}), and one treatment in which MPBs (1000 MPBs mL^{-1}) were incubated with Ag to promote adsorption. The presence of MPBs, at any of the tested doses, had no effect on the uptake or localization of Ag. However, exposure to the Ag-incubated MPBs (?75% of the Ag bound to MPBs) significantly reduced Ag uptake at both time points and also significantly increased the proportion of intestinal Ag. This study demonstrates that microplastics can alter the bioavailability and uptake route of a metal contaminant in a model fish species. A second study was undertaken to investigate the fate of Ag transported into the gut along MPBs. THis sencond study utilised in vitro gut sac preparations, but showed minimal differences in the internal fate of Ag regardless of its interation with the MPBs,

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Comparison of tissue preparation procedures to perform microplastic analysis; application to mussels (Mytilus edulis) from the Atlantic coast (Pays de la Loire, France)

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Plastics is a generic name to encompass most of the synthetic organic polymers exhibiting the property of plasticity. These products are characterized by many advantages which explain that they are massively used in our everyday life. Since the middle of the last century, several million tons of plastics have been produced. Ten percent of produced plastics is estimated to end up in the ocean. Once in the environment, macro debris undergo mechanical (erosion, abrasion), chemical (photo-oxidation, temperature, corrosion) and biological (degradation by microorganisms) actions. All these degradation processes lead to their fragmentation into microplastics (MPs) which accumulate in the environment. According to the last ten years of research, it appears that all natural habitats from pole to pole are affected by the presence of MPs. In these research works, many different procedures were used for MP quantification and characterization in environmental samples. Consequently, the aim of this study was to compare different tissue preparation procedures found in the literature to select the more appropriated. For that, counted MPs of two different forms (fragments and fibers) and types (PE and PVC) were added to three mussels (*Mytilus edulis*) for a digestion step implementing different solvents or mix of solvents. Then, the filtration step was performed using different types of filters. The efficiency of the digestion step was calculated from the masses of the filters related to the initial mussel masses and the preservation of the MP integrity was evaluated by using microscopy coupled to Raman and Infra-Red micro-spectrometers. The ratios were lower with the use of HNO_3 (65%) for the digestion. Nevertheless, the MP integrity was not preserved for all types of MPs added to the mussels since PE fibers were totally dissolved despite good recoveries for PVC and PE fragments. The determination of the better sample preparation procedure is quite difficult since it involves a compromise between efficient digestion of organic matter from mussel tissues and the preservation of the integrity of MPs. HNO_3 was however selected for the following analyzes since the lower organic matter amounts enhanced better microscopic and spectroscopic analysis. Finally this procedure was applied for the evaluation of MP contamination of mussels (*M. edulis*) from the Atlantic coast (Pays de la Loire, France).

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Methodological barriers for extraction and characterization of microplastics in biological matrix

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Pollution of the oceans by microplastics, defined as plastic particles of size below < 5mm by the NOAA, represent a major environmental problem worldwide. From an ecotoxicological point of view, the ingestion of microplastics by a wide range of marine organisms leading to substantial impacts on major physiological functions has been shown in several marine vertebrates and invertebrates. To date, only a few studies have investigated the levels of contamination of marine organisms collected *in situ*, partly due to technical difficulties in isolation and characterization of microplastics in biological samples. The crucial step is the identification of solvent(s) or chemical(s) that efficiently dissolve organic matter and biological tissues without degrading plastic polymers, and this in a time and cost effective way. Most published papers, as well as OSPAR recommendations for the development of a common monitoring protocol for plastic particles in fish and shellfish at the European level,used protocols containing nitric acid to digest the biological tissues, despite reports of substantial degradation of some polyamide types (also known as nylon) with this solvent. In addition, testing a wide range of plastic polymers, and especially those commonly found in the marine environment is essential to validate a common protocol and avoid an underestimation of plastic contents in marine organisms after tissue digestion. In the present study, we reviewed existing approaches and we compared (i) their efficiency in digesting biological matrixes and (ii) their effects on 5 different plastic polymers. Effects on plastics were evaluated through visual inspection, weighing and Raman analyses before and after digestion, while tissue digestates were filtered on 1μm-mesh fiber glass filters and observed using a binocular microscope. More research is currently ongoing in our laboratory on a wider range of plastic types (n=15 in total). The aim is to identify and validate a unique and standardized protocol that could be implemented at the international level to insure relevance and comparison of environmental studies on this topic.

Behavior Revised: Examining Behavioral Effects of Contaminants and Other Stressors in Aquatic Animals

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Why are avian focal species unaffected by chlorpyrifos applications? Integrating data on foraging-behaviour, exposure, and toxicokinetics

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Tier 1 avian risk assessment indicates high risk from use of chlorpyrifos (CP) due to its high toxicity to birds when tested in standard laboratory studies. However, when aiming to conduct a proper risk assessment, it is important to understand the status and ecology of the bird community present in the target crop at field level. In this respect, it is important to estimate basic population parameters of species breeding within the treated fields (long term survival and reproductive performance), their foraging behaviour and feeding ecology, and the spatial & temporal movement of the focal species to evaluate the factors (both natural and anthropogenic) which may influence reproductive performance and community of birds present. In addition it is equally important to collect exposure and mechanistic data to support and explain findings in the field. Several field studies have been conducted towards this aim. To gain information on the bird communities within the CP-treated fields a variety methods were used depending on the goal of the study and the species in focus, which allows measurement and ranking of any factors influencing the local bird community, including the application of CP itself. The work undertaken over consecutive years gave a good insight into exposure of birds utilizing treated fields/fruit orchards and showed a good correlation between the application of CP and cholinesterase (ChE) activity in the same bird communities, providing a diagnostic evaluation of CP exposure.

In order to support field findings and provide a higher tier risk assessment on birds, mechanistic information were also provided based on body burden model describing Toxicokinetics and Toxicodynamics. This presentation will describe the comprehensive and holistic approach which was followed, the new approaches for bird studies in risk assessment over consecutive years of data collection and will answer why the high risk of mortality predicted by the Tier 1 risk assessment is not seen in the field.

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Sex and steroids: Impact of a pervasive endocrine disrupting agricultural pollutant on sexual selection in fish

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Environmental contamination with endocrine disrupting chemicals (EDCs) threatens human and wildlife populations globally. The sub-lethal effects of endocrine disruptors at environmentally realistic levels are receiving increasing attention, including the impacts of exposure on behaviours influencing survival and reproductive fitness. Despite this, understanding of how these biologically active pollutants impact processes of sexual selection is rudimentary. In the present study, we investigated the effects of EDC-exposure on male mate choice, a key component of sexual selection and a driver of evolution by natural selection. Specifically, the aim of this study was to examine the impacts of short-term (21-day) exposure to an environmentally relevant concentration of 17b-trenbolone (measured concentration 6 ng/L)—an androgenic growth promoter used in the cattle industry globally that has been repeatedly detected in freshwater systems—on mate choice in the guppy (*Poecilia reticulata*). The guppy is a sexually dimorphic livebearer, with males inseminating females using their modified anal fin (the gonopodium) as an intromittent organ. Male guppies are choosy and prefer larger females as mates, and therefore gain fitness benefits as female guppy fecundity (brood size) increases with body size. Given that male mate choice is a key component of sexual selection, regarding not only whether to mate, but also the allocation of reproductive investment between each mate or mating, this male choosiness is key to reproductive fitness. To test the effect of exposure to 17b-trenbolone on male guppy preference for female size, we used a free-swimming experimental design, within which a pair of male and female guppies were tested in one of four treatments as follows: (1) unexposed male with 'large' stimulus female, (2) exposed male with 'large' stimulus female, (3) unexposed male with 'small' stimulus female, and (4) exposed male with 'small' stimulus female. We measured the time and frequency of male courting behaviours, as well as male structural colouration and morphology. The results of the mating behaviour trials, as well as colouration and morphological analyses, will be discussed with reference to the broader ecological and evolutionary consequences of EDC exposure.

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Effects of the NSAID Diclofenac on the survival, health and behaviour of embryonic and juvenile brown trout Salmo trutta f. fario

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While the presence of various pharmaceuticals of anthropogenic orgin in surface waters has frequently been shown, the ecological impact of the detected substances is still hardly assessable. Within the project "Eff-Pharm", new mode-of-action based *in vitro* systems for the monitoring of non-steroid anti-inflammatory drugs are developed. Simultaneous *in vivo* studies on diclofenac, a common and widely used representative of this pharmaceutical group, provide further information on possible effect concentrations. The brown trout has been investigated as a species of high local environmental relevance for Central European stream ecosystems. Our aim was to investigate two supposedly sensitive stages of trout life history: the embryonic development from fertilized egg until the end of the sac-fry stage, and juvenile fish around 6 months post hatch. The exposure of trout embryos and sac-fry stages was conducted according to OECD guideline 212, and included five test concentrations (0.1, 0.5, 1, 10 and 100 μg/L diclofenac) plus control - tested in triplicates for 127 days at 7 °C. Recorded endpoints were mortality, time until eye development, time to hatch, number of hatched larvae, heartbeat and histological condition of the fry. Juvenile trout (approx. 6 months post hatch) were exposed in a semi-static three-block design (control, 0.1, 1, 10, 100 and 200 μg/L diclofenac per block) for 25 days at 7 °C. Besides examining mortality, biometric parameters and behavioural abnormalities, additional biochemical analyses determined stress protein level. In addition, lipid peroxidation was measured and histological examinations were conducted in liver, gill and kidney tissues. None of the investigated parameters was altered in the embryos and fry. In contrast, juvenile fish showed increased mortality already at 0.1 μg/L diclofenac. Mortality rate was concentration dependent and reached up to 43 % in the highest test concentrations, while it did not exceed 10 % in the control. Furthermore, fish exposed to 10 μg/L diclofenac or higher showed a higher frequency of bite marks, a sign of increased territorial behaviour. Further histological analyses will give us a closer insight into the health state of the surviving individuals. Overall, the study showed alarming results on the toxicity of a commonly used NSAID. It also stresses out the importance of investigating different life stages to fully assess the potential effect of chemicals in the aquatic environment.

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Can zebrafish larvae behaviour be used for on-line contamination event detection in water distribution systems?

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Changes in motoric behaviour of organisms as a response to the exposure to chemicals are a sensitive endpoint for the assessment of toxicity [1]. Monitoring of zebrafish (embryo, larvae and adult) behaviour emerges as new, sensitive method to study effects of compounds in a vertebrate model. Here, we propose to use behavioural changes of zebrafish larvae for the detection of sudden contamination events in a water stream. Zebrafish larvae at the age of 96 hours post fertilization (hpf) were exposed to cadmium chloride (CdCl2) in a low concentration range and immediately monitored for avoidance behaviour which results in an increased activity. Here we propose two methods for the analysis of behaviour data and sensitive detection of alterations that have the potential to be for on-line data streams. For the first approach data on the distance larvae moved was pooled within groups (control n=16; 4 treatment groups at different concentrations with n=20 each) and averaged for one minute per group. In this approach two upper thresholds for maximal activity of the larvae during darkness were defined (180 mm/min and 200 mm/min). For the second approach the x- and y-coordinates of the larvae's movement where transformed to polar coordinates in order to calculate the angle of their movement. Thresholds for the angle allow distinguishing between undirected movement of the larvae and directed circular swimming behaviour that can be regarded as avoidance behaviour. First results show that the proposed approaches are suitable methods to detect alterations in zebrafish larvae behaviour in response to low concentrations of CdCl2. The thresholds presented in this study are sensitive with a minimum in false positive alerts and have the potential to be used for the analysis of an on-line data stream. The second approach considers the directed behaviour of the larvae and therefor allows for the detection of a distinct parameter that appears to be a sensitive indicator during the light cycles.

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The mechanism of oil sands process-affected water on feeding-behaviour toxicity in Daphnia magna

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Oil sands process-affected water (OSPW) is produced as a byproduct of the surface-mining oil sands industry in northeastern Alberta. Responsible remediation and returning OSPW to natural environment is a big concern of both oil-sand companies and scientists. Such a return would need to minimize the impact to downstream ecosystems. In our previous studies we showed that OSPW decreases the feeding activity of *Daphnia magna*. To understand the mechanism of OSPW's effect on feeding behavior we exposed *D. magna* to three substances [filtered OSPW (chemical portion), filtered OSPW plus added clay particles (whole OSPW), and clay particles suspended in solution (particulate portion)] and we used *Daphnia magna* culture water as an exposure control. We fed the animals similar quantities of their food algae, and gave them 24 h (in darkness) to graze. After 24 h we measured the food consumption in all treatments and the exposed *D. magna* were used for further experiments to understand the mechanism of effect of the components of OSPW on feeding activity of *D. magna*. For the behavioural bioassay, using a behaviour chamber designed for this study we examined the thoracic limbs and mandible beating as indicators of feeding rate of *D. magna*. We measured the activity of twodigestive enzymes (amylase and trypsin) as representatives of digestion of *D. magna*. Our results fromall studied biological levels showed whole OSPW significantly affects thoracic limbs and mandible movement, gut clearance, and digestive enzyme activity of *D. magna*. Particulate matter component of OSPW is most effective and chemical components are less effective in the observed reduction in the feeding behaviour of *D. magna*.

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Comparative behavioral toxicology of two common fish models

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Behavioral responses inform toxicology studies by rapidly and sensitively detecting molecular initiation events (MIEs) that propagate to physiological changes in individuals. Behavioral thresholds for a wide range of contaminants often manifest at lower levels than those eliciting mortality and other standardized adverse outcomes. Zebrafish larvae are common models in behavior, developmental toxicology, neurotoxicology and other biomedical studies. Whereas the fathead minnow is a common model for aquatic toxicology research and regulatory programs, they have received comparatively little attention in behavioral studies. We employed the zebrafish and fathead minnow models to define toxicant induced swimming activity alterations during interchanging photoperiods. We specifically examined behavioral response patterns among compounds specifically targeting various receptors/enzymes compared to response profiles for chemicals eliciting toxicity through narcosis. Following OECD FET and EPA WET experimental guidelines, zebrafish embryos and fathead minnow larvae were exposed for 96 h to each compound, then observed using a digital behavioral analysis system (ViewPoint). Behavioral observations occurred for 50 minutes (10 minutes acclimation, two 10 minute dark periods, two 10 minute light periods). In comparison to fathead minnow larvae, zebrafish generally displayed greater behavioral sensitivities to a number of the investigated compounds. Furthermore, zebrafish displayed more pronounced responses to changing light and dark photoperiods than the fathead minnow model. Such photomotor response profiles for compounds with defined MIEs are being used to examine industrial chemicals for which behavioral responses are poorly understood.

Fate, Effects and Risk Assessment of Chemicals in Aquatic and Terrestrial plants (I)

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Spatial and temporal distribution of pesticides and their metabolites in tomato plants

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Knowledge about translocation of plant protection products (PPP's) in plants is important to understand the uptake via the root system. Imaging mass spectrometry (IMS) has been widely used in life science and has proven to be a powerful tool to combine histological data with specific molecular information. So far phospholipids, peptides, drugs and plant metabolites were imaged in a number of tissue samples at a spatial resolution of 5–10 μm[1-4]. The IMS data for all these experiments showed excellent correlation with histological staining evaluation. Thus, IMS allows exploration of both PPP's and their metabolites distribution in plant tissues and provides new knowledge concerning behaviour during plant growth. Within a single experiment two-dimensional spatial maps of parent compound and metabolites can be generated. We report here the use of a matrix-assisted laser desorption/ionization (MALDI) imaging source working at atmospheric pressure which is coupled to an orbital trapping mass spectrometer for new insights into molecular processes inside the plants. Therefore, spray application onto the tissue using selected matrix solution has to be optimised, supporting desorption and ionization of target compounds by means of a focused laser. This sample preparation is important to improve sensitivity and spatial resolution of the IMS-measurement and finally to generate the images. Here, we report the use of MALDI-high resolution-IMS to map the distribution of two selected PPP's (the phenylamide fungicide metalaxyl and the triazole fungicide tebuconazole) and their metabolites in roots, stem and leaves of tomato plants in high-spatial resolution with minimised sample preparation. The combination of autoradiography of ^{14}C-labelled compounds, LC-HRMS^n, and MALDI-IMS provides comprehensive information of distribution, localization and dynamics of PPP's uptake via the root system of tomato plants.

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Toxicity testing with the willow tree transpiration test - 15 years of results

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Due to the large size of trees, their slow growth and the related difficulties, few toxicity tests for trees have been developed, and no standard test is available. A toxicity test using the transpiration of willows is in use since 15 years [1]. The willow tree toxicity test was employed for toxicity testing, for phytoremediation purposes and for process studies in the soil-plant system. Genetically homogenous willow cuttings are grown under artificial light in 500 mL Erlenmeyer flasks filled with spiked nutrient solution (ISO 8962), contaminated soils, wastewater or other test substrate [1]. The toxic endpoint is the change of transpiration, which is measured daily by weighing the whole flask, compared to the transpiration of control trees. **\n** EC50-values of more than 50 compounds obtained under various conditions. EC50-values < 1 mg/L were obtained for Cu(2+). Cd(2+), As (arsenate) and Se have EC50 close to 1 mg/L. Among the pharmaceuticals tested, the most toxic was the weak acid ibuprofen with an EC50-value of 1.7 mg/L, but only at pH 4, and much less toxic at higher pH. Diclofenac (100 mg/L) and paracetamol (3000 mg/L) were less toxic in this test. The bivalent base chloroquine had an EC50 of 2.5, 10, 35, 40 mg/L at pH 9, 8, 7, 6, respectively [2]. The toxicity of chlorophenols declined from 1.1 mg/L (3,5-DCP) to 5.8-10 mg/L (2,4-DCP) to 32-84 (4-chlorophenol) and 400 to 800 mg/L (phenol). The toxicity of cyanide depended very much on the complexation, from rather non-toxic 1000 mg/L Prussian blue (ferriferrocyanide, FeII,FeIII-complex) to quite toxic with EC50 at 2 mg for KCN. Toxicity to willows grown in sand was generally lower, for example, EC50 was 20 mg/L for KCN in sand. Little toxicity (contrary, growth stimulation at lower dosage) was observed for nano-TiO2 (> 100 mg/L) and zero-valent iron nanoparticles (around 1000 mg/L) in solution. A user manual has been developed and is freely available (http://homepage.env.dtu.dk/stt/). Overall, the willow tree test is probably the most widely used toxicity test with trees and could be seen as a candidate for a standard toxicity test for woody terrestrial plants. [1] Trapp S, Zambrano KC, Kusk KO, Karlson U. 2000. A phytotoxicity test using transpiration of willows. Arch. Environ. Contam. Toxicol. 39(2):154-160

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Uptake of pharmaceuticals from wastewater into plants - Novel carbamazepine metabolites in tomato plants grown under hydroponic conditions

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Water scarcity is one of the main challenges in arid and semiarid regions. Therefore, the use of treated wastewater (TWW) for crop irrigation is a necessity. Under these circumstances pollutants of emerging, with low removal efficiency in wastewater treatment plants, are introduced into the agro-ecosystem and may be transferred from irrigation water into edible plants and thus enter the food chain [1-3]. However, until now little work has been done to investigate the metabolism of PPCPs after uptake into plants. We studied the uptake and metabolism of PPCP from wastewater into plants with carbamazepine (CBZ) as model compound, an antiepileptic drug which is a commonly detected pharmaceutical in wastewater, surface water and even groundwater [4]. Tomato plants grown in hydroponic cultures were analyzed with both targeted analytical methods (LC-MS/MS) that offer higher sensitivity and liquid chromatography-high resolution mass spectrometry (UPLC-Q-TOF-MS) to identify unknown metabolites. A total of 10 phase-I and three phase–II metabolites could be detected from the one contaminant CBZ. Considering these known CBZ metabolites the overall concentration was about 1.5-fold (for tomato leaves and fruits) higher than that of CBZ alone. This example illustrates that, due to plant metabolism, the concentration of contaminants found in plants can be significantly higher than the concentration of the parent compound. However, further study is needed to evaluate the potential toxicological effects of these metabolites and the consequences for the safe reuse of treated wastewater in agriculture. In addition, further research is required to better understand and possibly to predict the metabolism of compounds within plants in terms of potential consequences for modelling. References: [1] Goldstein M, Shenker M, Chefetz B. 2014. Environ. Sci. Technol. 48:5593-5600. [2] Macherius A, Eggen T, Lorenz W, Moeder M, Ondruschka J, Reemtsma T. 2012. Environ. Sci. Technol. 46:10797-10804. [3] Shenker M, Harush D, Ben-Ari J, Chefetz B. 2011. Chemosphere 82:905-910. [4] Ternes TA, Bonerz M, Herrmann N, Teiser B, Andersen HR. 2007. Acknowledgement: Financial support of this study by the Deutsche Forschungsgemeinschaft (DFG, Bonn) through the project PECtake (Re1290/7-1) is gratefully acknowledged.

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Aquatic macrophyte modelling - Increasing the realism of risk assessments

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For the registration of plant protection products, the risk on aquatic macrophytes has to be assessed. This is done by predicting the annual concentration pattern of a plant protection product in several surface water systems considering its specific agricultural application. To assess the risk on aquatic macrophytes, as a first step, the maximum concentration that is predicted throughout the year is compared to toxicity endpoints, such as EC50 values. In most cases, these toxicity endpoints are based on studies considering constant concentrations of the respective plant protection product. A method to increase the realism (higher tier) of the risk assessment is to use toxicity studies that are based on exposure patterns similar to those predicted in surface water systems. To date, this approach is mostly applied to short term exposure patterns, having a duration of less than a week, due to the time range of standard macrophyte toxicity studies. This contribution demonstrates ecological modelling methods, in terms of toxicokinetic and toxicodynamic (TK/TD) growth models of *Lemna* spp. and *Myriophyllum spicatum* that quantify adverse effects caused through entire and complex exposure patterns. Special emphasis is on the toxicity data needed to reliably calibrate the models. Modelling results showed a broad variation of predicted effects strongly depending on the specific exposure patterns of a plant protection product. Another focus of this contribution is the presentation of semi-field growth data *of M. spicatum*. The monitoring of plant growth was initiated to identify the spatial capacity limit of plant growth and to obtain long-term growth data that are otherwise hardly available for aquatic macrophytes. The maximum capacity of total plant length was approximately 150 m for the containers with their specific spatial dimensions.

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Plant community modelling as a means to assess herbicide effects on plant reproduction using the IBC model approach

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Risk assessments of plant protection products include testing effects on non-target terrestrial plants (NTTPs). To address the protection of plant communities at landscape level, several researchers state that the full plant life cycle should be assessed, since reproductive endpoints (e.g., seed production and germinability) may be more relevant for the continuation of the populations. It remains unclear, which effect extent might be tolerable to still preserve plant populations. During a follow-up SETAC workshop on NTTPs held in Wageningen on September 21 and 22 2015, the need for reproductive studies was discussed and extrapolation from existing studies appears meaningful. The framework of Xplicit-IBC offers an excellent opportunity to address this task. It aims to analyze herbicide drift effects on non-target terrestrial plant communities and populations within a landscape community context by utilizing an individual-based spatiotemporally explicit plant community model (IBC-grass). IBC-grass is an individual-based stochastic plant community model for grasslands using the plant functional type approach to cover a wide range of species. It was parameterized for a typical herbaceous field boundary community in Europe. The regional species pool was based on an extensive literature research and was classified into plant functional types (PFTs). Due to the individual-based approach of IBC-grass, we were able to address potential herbicide effects on relevant reproductive attributes of plant individuals. In a first model test phase, we conducted unrealistic worst-case simulation experiments in which we differentiated the relevant attribute(s), effect extents and the affected PFTs. These simulations indicate that effects on reproductive endpoints can have complex impacts on PFT populations. Individual-based plant community modelling can provide valuable tools to clarify the need and - if necessary – the design of future experiments. Further investigations will analyze in greater depth the dependency of effects on plant trait characteristics (e.g. type of reproduction) and the sensitivity of potential endpoints. In these studies herbicide exposure will be simulated in a more realistic pattern to investigate real-world situations (e.g., variable herbicide exposures) and will be upscaled to a landscape level by integrating the herbicide exposure model Xplicit.

Metals in the Environment: Fate, Speciation and Bioavailability in Water, Soil and Sediment (I)

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Modelling chromium(III) binding to humic substances and its speciation in soil solution

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Chromium exists in natural environments in the Cr(III) and Cr(VI) redox state. Humic substances can reduce the more toxic Cr(VI) to Cr(III). Cr(III) binds very strongly to organic matter in soils which reduces its availability and protects it against oxidation. Binding of Cr(III) to humic substances is however hardly quantified and binding constants for Cr binding to humics in ion binding models are based on very limited experimental data. Modeled Cr(III) speciation in soil solution using the NICA-Donnan model in combination with generic model parameters deviated strongly from speciation measurements in soil solution extracts with the Donnan Membrane Technique (DMT). To quantify Cr binding to humics we studied the binding of Cr to an isolated fulvic and humic acid using the DMT. Based on these results new model NICA-Donnan model parameters for Cr binding to humics were derived, using the coupled PEST-ORCHESTRA modeling tool. Additional information other than macroscopic adsorption data were nescessary to make scientifically sound choices with respect to the parameterisation.

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Understanding the behaviour of silver in surface and wastewater environments: Experimental validation of WHAM

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Exposures to ionic silver are known to result in adverse effects in laboratory test organisms at concentrations in the low parts per billion range. Yet, environmental exposures are rarely in the ionic form and so are less ecotoxicologically relevant. It has long been established that dissolved organic carbon (DOC) and sulphur compounds in freshwater and effluents can bind with silver mitigating ecotoxicity and influencing the behaviour and fate. Concentrated samples of natural organic materials were collected from four freshwater sites in Canada using reverse osmosis. Standard samples of natural organic matter (NOM) concentrates, collected using a similar reverse osmosis method to that employed for the field samples, were also obtained from the International Humic Substances Society (IHSS). The sludge from a raw sewage sample was also tested as a binding matrix.Silver binding to the organic matter concnetrates, including raw sewage, was determined by titration using a silver ion selective electrode. Organic matter samples showed varying levels of silver binding at the same metal to carbon ratios. Various properties of the organic matter samples were determined in order to identify the factors affecting silver binding strength between different samples. The properties investigated included fluorescence, specific UV absorption, thiol concnetrations, and chromium reducible sulfide concentrations. Chromium reducible sulfide concentrations in the organic matter samples correlate with the degree of silver binding and may be used to refine WHAM predictions of silver speciation.

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Electroanalytical techniques to determine free concentrations and lability degrees in Indium solutions.

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Indium is a critical element present in electronic devices, from which it can eventually leach to natural waters. The large hydrolysis processes of Indium (e.g. an increase by 0.1 units in pH in precipitated systems decreases the free concentration by a factor of 2) hinder the accurate study of its speciation and availability with most conventional techniques and, so, there are many unresolved aspects of the behaviour of free Indium in various systems[1]. AGNES (Absence of Gradients and Nernstian Equilibrium Stripping) is an emerging electroanalytical technique designed to determine free metal ion concentrations in solutions, such as seawaters, river waters, dispersions of nanoparticles, extracts of soils, etc (see recent review[2]). The implementation of AGNES with mercury electrodes requires amalgamating elements such as Zn, Cd, Pb, Sn or In. Due to some irreversibility of Indium on the Hg electrode, a new calibration strategy has been developed. Speciation results in the system Indium+Nitrilotriacetic acid with AGNES agree with computations using database NIST 46.6. However, the evolution of free Indium for increasing amounts of the ligand oxalate indicates that the values of the stability constants in NIST 46.6 [3] are not so accurate as those of Vasca *et al*.[4]. A new strategy, called Accumulation under Diffusion Limited Conditions (ADLC), can be applied to compute the lability degree of the complexes. The lability degree is strongly linked to the availability of this element as it describes the contribution of the complexes to the overall uptake flux. The use of highly labile complexes (such as those of oxalate) allows for a dramatic reduction of the deposition times, especially for huge gains (i.e. preconcentration factors). For instance, in the system oxalate+In at pH 3, equilibrium for a gain larger than 300000 was attained in just 25 seconds retrieving $[In^{3+}]=3.71\times10^{-10}$ M. In precipitated solutions at pH 5.6 (where the free Indium concentration is fixed despite the addition of the helping oxalate), $[In^{3+}]= 1.87\times10^{-11}$ was determined. References: (1) Tuck, D. G. *Pure Appl.Chem.* **1983,** *55*, 1477. (2) Galceran, J. et.al. *J.Electroanal.Chem.* **2014,** *722-723*, 110. (3) Pingarron, J. M. et.al. *Bulletin de la Societe Chimique de France* **1984,** *3-4*, 115. (4) Vasca, E. et.al. *Dalton Trans.* **2003,** 2698.

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Colloidal metal speciation in historically contaminated sites studied using centrifugal FFF

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Many indications exist that natural colloids are often vectors for many metals, predicting large discrepancies between modelled and experimental results, particularly for those metals having relatively high partitioning coefficients (K_d values), such as lead (Pb). Centrifugal Field flow fractionation (cF^3)and sequential filtration wereused to analyse the colloidal speciation of several metals released during unsaturated leaching of intact cores of historically contaminated sites in an irrigation chamber. It was also investigated whether this speciation changes during simulated extreme rainfall events. cF^3 ♀_ ICP-MS complemented sequential filtration data the former technique gave a much more detailed picture of the association between several elements simultaneously. Most metals of interest were found as dissolved species and/or associated with relatively small colloids (< 200 nm). The speciation of lead was spread over iron oxides, organic matter and an aluminium bearing phase, but it was unclear whether the latter phase was a clay fraction or aluminium oxides. Sb occurred in relatively larger particles only in one particular soil from an industrial area without associations with other elements. As and Cr appeared mainly dissolved, but partly associated with iron oxides. Differences between the two rainfall scenarios were, in general, limited. The division of metals over dissolved phase and differently size colloids has implication for modelling their fate and bioavailability in realistic systems,

because K_d values may not always provide the most accurate prediction. The relative importance of a kinetic *versus* thermodynamic approach is discussed.

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Specific parameterization of WHAM improves the prediction of copper competitive binding on plant roots

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The relevance of the default humic acid (HA) in the Windermere humic aqueous model (WHAM) for modelling the binding of metal cations such as copper exhibiting high affinity for plant roots is questionable. We thus compared the ability of the default parameterization of WHAM and a specific parameterization for terrestrial higher plants (WHAM-THP) to model the competitive binding of copper on wheat (*Triticum aestivum* L.) and tomato (*Solanum lycopersicum* L.) roots. WHAM overestimated by twofold the binding of copper on roots under varying ionic strength and concentration of competitive cations (proton, calcium, and zinc). With a single set of parameters for both wheat and tomato, the specific parameterization of WHAM-THP improved the goodness of the fit of copper competitive binding ($\log_{10}$ of the root-mean-squared residual = 0.15) without any systematic bias. We put forward proposals for the practical application of WHAM-THP in predictive ecotoxicology.

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Speciation and localisation of gadolinium in root tissue of Zea mays

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The group of rare earth elements (REE) consists of 17 economically critical elements, the lanthanides and Sc and Y. Their physico-chemical properties make them useful for many high tech applications. The lack of economic recycling ways might result in the accumulation of REE in the environment, which might negatively affect organism like plants as primary producers. Bioavailability of REE was shown to be high, especially for fine ground materials like tailings [1]. Earlier studies [2] also showed high concentrations of Gd in the root tissue, with low transport to shoot tissue, therefore, speciation and localisation of Gd in root tissue was conducted. Tof-SIMS, as a surface analytical technique, offers high sensitivity and can generally detect all elements and isotopes. Gd was chosen for a hydroponic solution culture using a culture crop *Zea mays* (cv. Ronhaldino). It was applied in a concentration of 10 mg L^{-1} as nitrate. The plants were grown in a greenhouse in eastern Germany during the warm and sunny month April. After 14 days the plants were harvested. The roots were used for the investigations presented here. Thin sections ranging between 8 and 20 μm were analysed for the location and species of Gd by ToF-SIMS (ION TOF). Total concentrations of dried bulk samples were measured by ICP-MS (Agilent 7700) after digestion. High content of Gd was found in the dried bulk samples of the roots after aqua regia digestion, 8.43 ± 0.95 g kg^{-1}. Gd was analysed in spots of high concentrations at the epidermis of the root. The typical isotope pattern was identified for Gd as well as for Gd species bound to oxygen. The expected Gd-phosphates could not be found in these spots. The occurrence of the spots in the outer epidermis might be an explanation for the low transfer rates. For the first time, ToF-SIMS analysis with a spatial resolution of 4 μm was used to locate these spots and give information about the species of the Gd in the plant root. 1. Mittermüller, M., Saatz, J., and Daus, B., *A sequential extraction procedure to evaluate the mobilization behavior of rare earth elements in soils and tailings materials*. submitted. 2. Saatz, J.,Vetterlein D. Mattusch, J. Otto, M, Daus, B., 2015, *The influence of gadolinium and yttrium on biomass production and nutrient balance of maize plants*. Environ Pollut,. 204: 32-8.

Natural toxins: an on-growing challenge for environmental research, monitoring and management

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Cyanobacterial toxins: from genome mining to risk assessment

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Cyanobacteria are photosynthetic prokaryotes proliferating in many ecosystems worldwide. This phylum is also known to produce a wide range of bioactive natural products, notably cyanotoxins linked to animal deaths and human health hazards through drinking and recreational waters. Cyanobacterial natural products are complex peptides, polyketides and hybrid metabolites such as the well-studied hepatotoxin microcystin. Current strategies for cyanotoxin-related risk assessment are based on detection of the known producers or of the already characterized toxic compounds. This strategy does not take into account the larger cyanobacterial potential recently uncovered with genomic and metagenomic as well as metabolomic analysis. Furthermore, emerging toxins are not monitored or regulated. We studied the bloom-forming genus *Planktothrix* genetic potential dedicated to the biosynthesis of natural products. Diversity and distribution was established by *in silico* genomic analysis of newly sequenced isolated strains of diverse origin. Natural products were also characterized by mass spectrometry analyses to detect variants of the known peptides and novel natural products of this cyanobacterial genus. This multidisciplinary analytical approach could be broaden and extended with molecular biology tools for early detection of toxic potential of HABs in environmental samples. Most of the time even in the case of known compounds such as the toxin microcystin, the cellular and ecological function of the metabolite remains unknown. The understanding of regulation of natural product synthesis, as well as the cyanoHABs internal population regulation is only at its first steps. The use of genome mining strategies on NGS environmental and experimental data will lead to a more systematic survey of potential hazards. Although challenging, closing this knowledge gap between producers and their genetic potential, regulation and metabolites detection will give rise to a better risk assessment in freshwater ecosystems.

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Structure-dependent effects of selected cyanobacterial peptides on innate immunity

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Cyanobacteria produce many biologically active metabolites synthesized via nonribosomal synthetic pathways. These are classified into several families, according to their structural features, and include also cyclic microcystins (MCs) and linear aeruginosins (AURs). Although MCs are toxicologically known for their hepatotoxic effects, their immunomodulatory potential is not known. In contrast, aeruginosins have been studied in much lesser extent but recently isolated new aeruginosin-865 (AUR865) showed some anti-inflammatory potential. The present study aimed to investigate in detail the effects of three different MC variants and AUR865 on macrophages, which represent one of the key effector cells within the innate immune responses. Specifically, our study includes investigation and comparison of several structurally different cyanobacterial peptides on macrophage activation, associated with production of cytotoxic and cytostatic products such as nitric oxide (NO), as well as proinflammatory mediators (e.g. tumor necrosis factor α, TNFα and interleukin 6, IL-6). Although MC-LR was able to significantly affect the macrophages, other most common MCs (-RR, -YR) and AUR865 did not alter immune responses. Our recently published results on affected molecular pathways (doi: 10.1021/acs.est.5b02049) provide an interesting mechanistic explanation of some adverse health outcomes associated with toxic cyanobacteria. The research was supported by the projects of the Czech Ministry of Education LO1214 and LM2011028, and by the CYANOCOST action (EU COST ES1105).

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Occurrence of emerging marine toxins in coastal areas of the Catalan coast

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Marine toxins are natural substances synthetized by phytoplankton and other organisms. Main concern about these compounds is due to its potential toxics effects, especially when a harmful algal bloom (HAB) takes place. During this phenomenon, high amounts of toxins are generated; its accumulation in the particulate can favour the eutrophication of the waters and on the other hand, zooplankton, fish and shellfish that can suffer intoxication with poisoning or lethal effects. Moreover, it has been evidenced that the concentration of toxins can bioaccumulate along the food chain, supposing a potential risk for human health. Monitoring of these compounds has become an important issue during last decades. New analytical methodologies are required with the aim of determining the distribution, transport and behavior of marine toxins with enough accuracy and sensitivity. In this study, an analytical methodology has been developed and validated for the detection of different families of the most frequent marine toxins in seawater and estuarine areas. This method is based in solid phase extraction (SPE) followed by high performance liquid chromatography (HPLC) coupled to high resolution mass spectrometry (HRMS). In order to assess the occurrence and distribution of these marine toxins, the developed methodology has been applied in real samples from the western Mediterranean Sea and the Ebro's delta basin. Samples were collected in the same areas for different periods of time in order to characterize the occurrence of marine toxins in different seasons. Preliminary results show the presence of some marine toxins in similar concentration for all seasons and in non-algal bloom conditions. These results highlight the importance of further studying the degradation patterns, distribution and chronic toxic effects of these substances in order to properly carry out a risk assessment.

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Characterization of the voltage-gated sodium channel and its isoforms in the Pacific oyster Crassostrea gigas: link with its sensitivity to paralytic shellfish toxins produced by Alexandrium minutum

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French coasts are regularly affected by blooms of the dinoflagellate *Alexandrium* spp. These micro-algae can produce paralytic shellfish toxins (PST), such as saxitoxin (STX) and its derivatives, which bind to voltage-gated sodium (Nav) channels and block conduction of action potential in muscle and nerve fibers. Filter feeder marine organisms, such as oysters, can accumulate high levels of PST, making them toxic and unsuitable for human consumption. The toxin load in *Crassostrea gigas* oysters, has been shown to vary between individuals, sampled from a same population and then exposed experimentally to *A. minutum*. These results suggest a high variability in PST tolerance. The purpose of our study was (i) to characterize Nav channel and its isoforms in *C. gigas* and (ii) to determine whether Nav channel isoforms play a role in the sensitivity of *C. gigas* to PST. The tissular and cellular expression pattern of Nav was characterized by real-time PCR and *in situ* hybridization. Nav appeared mainly expressed in neurosecretory cells of the visceral ganglia, in nerve fibers of the striated muscle and in epithelial cells of the labial palps and mantle. First sequencing analysis of Nav cDNA in muscle and visceral ganglia revealed three alternative splicing transcripts. These transcripts have a specific tissular expression. Only one transcript is found in the striated muscle, whereas the two other transcripts are expressed in the visceral ganglia. This alternative splicing could be a source of the differential sensitivity to PST in *C. gigas*, as demonstrated in some insects for resistance to pyrethroids, and consequently could lead to variable toxin load in oysters exposed to *A. minutum*. As a result, we will analyze the presence/absence of each splicing variant as well as their expression level in muscle and visceral ganglia in oysters experimentally exposed to *A. minutum* to test the correlation between Nav variant expression and toxin accumulation.

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Identifying phytotoxins of environmental concern
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Today, environmental scientists, regulators and decision makers are well aware of the presence and potential risk of anthropogenic chemicals in the aqueous environment. In contrast, natural toxins from plants (phytotoxins) have not yet been perceived as potential aquatic micropollutants. A number of arguments lead us to hypothesize that phytotoxins might indeed play a role in this respect, the most important of which are: 1) there are thousands of toxic plants, containing a variety of even more chemically very diverse phytotoxins, 2) exposures in anthropogenically managed/affected (agro-)ecosystems can be orders-of-magnitude higher than in the pristine environment, 3) phytotoxins are toxic by definition, although their mode of action is often unknown, 4) effects of phytotoxins (on non-target organisms) have been shown in case of allelochemicals, invasive plant species, and biopesticides, and 5) to properly evaluate the risks of (emerging) anthropogenic contaminants, the background stress/toxicity potentially exerted by natural toxins should be known. Therefore, we propose a systematic assessment of the aquatic environmental exposure to phytotoxins in Switzerland. This is accomplished with a sequence of iteratively refined work packages, including: 1) compilation of a database of phytotoxins, based on the spatial distribution and chemical fingerprint of toxic plants in Switzerland, 2) elucidation of physicochemical properties of phytotoxins using literature data or in silico estimations, 3) prediction of the phytotoxins' environmental fate and behavior and likely recipient matrices by means of in silico multi-compartment mass-balance models, 4) surface water sampling in collaboration with respective monitoring networks, and 5) target and suspect analysis of phytotoxins in the collected water samples. This way, we aim to identify among the many thousands of phytotoxins those – probably rather few – that actually may be considered as aquatic micropollutants.

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Environmental fate and ecotoxicological impact of leptospermone, a natural β-triketone herbicide in soils
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Most agricultural production relies on the use of chemicals to maintain high crop yields. The use of these chemicals in farming practices is viewed as an integral part of the success of the intensive farming. However, most of the pesticides applied to agricultural lands may affect non-target organisms and contaminate soil and water media. Increasing public concern about the impact of pesticides on the environment, and European legislation has led to develop some strategies to evaluate and to prevent the potential impacts of different land management practices. In this context, new generations of less environmentally dangerous molecules, such as lower impact pesticides, biopesticides and natural product-based pesticides have been introduced. Despite this, a lot of work has to be carried out to evaluate the possible risks and adverse effects of such compounds on environment and humans. In the frame of the TRICETOX project, we study the β-triketone herbicide family, a post-emergence maize selective herbicides belonging to this new generation of molecules. Inside this family, we focus on leptospermone, an allelopathic compound isolated from the bottlebrush plant *Callistemon citrinus*. Since few data are available for this molecule, we want to gain new insights by addressing its soil behaviour, by studying its impact on soil microbial communities, by studying its biotic and abiotic degradation and by testing its toxicity. Then, multidisciplinary approaches were applied to assess the fate and impact of leptospermone in two different soil types. Our results showed that in the two tested soils, leptospermone has a similar fate to that of the synthetic β-triketone herbicides, such as sulcotrione or mesotrione. Interestingly, leptospermone exhibits a 10 fold lower toxicity, determined with Microtox^R than the two synthetic counterparts. Although leptospermone was rapidly dissipated from soil, it has a significant ecotoxicological impact on the abundance and diversity of soil bacterial community. This ecotoxicological impact was transient and when leptospermone was fully dissipated, the bacterial community recovered its initial composition and diversity. However, the recovery cannot be reached in soil microcosms where leptospermone remained. Characterization of a bacterial isolate able to degrade leptospermone is in progress. Further work need to be done to test the ecotoxicological impact of this natural β-triketone on a wider range of soil classes.

Advances in exposure modelling: bridging the gap between research and application (I)

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Screening level Environmental Exposure modelling Of Engineered Nanoparticles: An analysis using SimpleBox4nano
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In order to sustain the development of nanomaterials by industry, a robust risk assessment framework is needed. A screening level environmental exposure model is one of the first steps of such a framework currently in place in Europe and the United Sates, but this needs adaptation for use with nanomaterials . For this reason, SimpleBox4nano was developed as a first step in developing a screening level exposure model that can be used as part of such risk assessment frameworks currently in place for conventional chemicals. Here we present a further analysis of the SimpleBox4nano model in order to continue its development towards a robust screening level exposure assessment model for nanomaterials. First an analysis is done of the uncertainty and variability in output exposure concentrations of three metal oxide ENPs (TiO_2, ZnO and CeO_2) in relation to the uncertainty and variability of the emission rates and a broad selection of input parameters, including environmental properties such as e.g. ambient particle concentrations and temperature. Second, a sensitivity analysis is done for the nano-specific properties: nanoparticle size, density, dissolution rate and attachment efficiency while still accounting for variability of the environmental properties in order to more clearly understand the significance of these specific nanomaterial properties compared to natural variability.

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A Kinetic Environmental Fate Model for the Risk Assessment of Engineered Nanomaterials
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An integrated exposure model is proposed for the prediction of the environmental fate of nanomaterials (NMs) in various natural and man-made compartments. A semi-mechanistic approach is used, wherein the kinetic nature of the NM fate processes takes focus. Environmental matrix interactions depend on NM properties and relevant parameters, such as organic matter and ionic conditions, and are calculated on a temporal scale in each compartment. The risk quotient is calculated based on the maximum calculated temporal NM bioavailability and existing hazard information. The goal of this approach is to apply the model for most currently-produced and possible future NMs, also aiming to find a compromise between mechanistical accuracy and operational simplicity. In the current model, fate descriptors are detailed for the following compartments: soil, surface water, sediment and wastewater treatment plant (WWTP). The WWTP is the main entry point for NMs emitted with domestic and industrial liquid waste. Partitioning of NMs in the WWTP, based on their affinity to sludge, determines the amounts that are transported to the surface water (in the effluent) and the soil (via sludge). The physicochemistry of the surface waters, including fresh-, estuarine-, and marine water, and the properties of the NM are among the factors

that affect its bioavailable concentration in the water column and, after possible sedimentation, in the sediment within a certain time. The kinetic model includes rates of the following key processes: dissolution, sulfidation, heteroaggregation, and sedimentation. NMs arrive in topsoils via the annual deposition of sludge. The time-dependent concentration in the pore water (liquid phase) and on the soil material (solid phase) is determined using a dual deposition model, allowing both NM release and (ir)reversible attachment. The time-dependency of chemical species concentrations is essential since it evokes realistic time-dependent exposure scenarios whereas available hazard values are almost always considered static. This information is relevant in assessing the bioavailability and eventual risks of NM release and transport. This kinetic fate model is a key component in the web-based tool for the assessment and management of risks associated with NM-enabled consumer products, under the GUIDEnano Project (EU FP7). This work also contributes to the NanoFASE Project (EU H2020).

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The importance of temporal variables in environmental modelling: Rationalizing measured concentrations of cyclic volatile methyl siloxanes in an Arctic lake

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Cyclic volatile methyl siloxanes (cVMS) are used in personal care products and are emitted to aquatic environments through wastewater effluents. In this study, we describe a holistic evaluation of the environmental fate of cVMS in an Arctic lake (Lake Storvannet, 70 °N 23 °E) experiencing seasonal variations in environmental conditions and intermittent wastewater emissions. Sewage, lake water, and sediment samples were collected and analyzed for cVMS concentrations in 2014. While measured cVMS concentrations in lake water were below detection limits, concentrations in sediments were comparable to concentrations observed in higher populated areas. To rationalize measurements, Mackay et al.'s QWASI-model was parameterized for Storvannet and run for steady-state conditions assuming constant emissions and under dynamic conditions accounting for temporally variable emissions, water-flows, temperatures and ice-cover. Inverse modelling was employed using the steady-state QWASI to estimate the average rate of emission from the measured concentrations of cVMS in sediment. This resulted in a significant over-prediction of cVMS concentrations in water compared to actual measurements and to unrealistically high emission estimates to the lake based on measured concentrations in sewage. The dynamic model could mechanistically rationalize high (and relatively stable) concentrations of cVMS in sediment and low concentrations in the water column by employing a hypothetical intermittent emission scenario consistent with combined sewer overflows and a lower annual average emission. In both the steady-state and the dynamic scenarios, the most important removal process for cVMS from lake water was predicted to be advection due to the high rate of water-turnover, while removal processes typically dominant in other aquatic systems were limited in Storvannet due to low temperatures, ice-cover and slow sedimentation rates. This study illustrates the benefits of combining monitoring with models as interpretive frameworks to understand the environmental behaviour of organic contaminants, and the need to balance simplicity and complexity in modelling efforts. In addition, the insight gained on the fate of cVMS in Arctic environments may be useful for interpreting biotic exposure in Arctic environments and may inform ongoing regulatory debates for cVMS materials.

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Can eutrophication impact air-sea exchange and concentrations of contaminants in marine systems?

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Eutrophication impacts not only biogeochemical and ecological processes in many water bodies, but potentially also the distribution and degradation of many organic contaminants since these substances have a tendency to partition into organic phases. A number of hypotheses for interactive mechanisms between contaminants and eutrophication have been postulated. One important mechanism is the removal of chemicals from the atmosphere via the so called biological pump. How the importance of this and other processes related to the marine carbon cycling varies with the inherent properties of the chemicals has, however, yet not been evaluated. In this study we use a modeling tool with multi-stressor functionality (Baltsem-POP) to assess how changes in nutrient input and the subsequent changes in primary production may influence concentrations and total input of organic contaminants in marine systems such as the Baltic Sea. The average concentrations and fluxes of a wide range of organic contaminants in the Baltic Sea by the end of this century were predicted to differ by at most a factor of 2 when comparing a plausible future eutrophic and a more oligotrophic system. Whereas eutrophication increased or did not change total input from the atmosphere for all compounds, dissolved concentrations in the eutrophic system were always lower or not impacted for all modelled chemicals. The predicted chemical mass stored in the active sediment layer was not consistently positively or negatively influenced by increased primary production across the chemical space, instead reflected the combined influence of increased atmospheric input and depleted surface water concentrations. We found that mitigation of anthropogenic eutrophication may increase dissolved concentrations of many compounds with relatively low volatility in surface waters, but at the same time lower the total input from the atmosphere of more volatile hydrophobic contaminants and consequently their accumulation in sediments.

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Integration of environmental and human PBPK exposure models: application of MERLIN-Expo modelling tool to POPs exposure in Venice lagoon.

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MERLIN-Expo is a new tool for integrated exposure assessment recently developed under the FP7 project "4FUN". MERLIN-Expo is a simulation platform providing a library of multimedia and physiologically-based pharmacokinetic (PBPK) models which can be coupled in order to estimate ecological and human exposures in complex scenarios. Models can be used to simulate fate of organic (PAHs, PCBs) and inorganic contaminants. Software enables end-user to apply set of functionalities such as uncertainty and sensitivity analysis, dynamic deterministic and probabilistic simulations in order to address different exposure and chemical fate problems. MERLIN-Expo was applied to assess the ecological and human exposure to PCBs and PCDDs in the Venice lagoon. The Phytoplankton, Aquatic Invertebrate and Fish models were developed and implemented in MERLIN-Expo library, subsequently integrated to create specific aquatic food web to dynamically simulate bioaccumulation in different aquatic species. Concentrations of PCBs and PCDDs in water were used as time-dependent inputs to run long term simulations. The estimated concentrations in edible aquatic species were then used to estimate daily human intake through the consumption of local seafood. The application of the PBPK model allowed to explore the time dependent accumulation of target chemicals in human tissues for several decades. Modelling results are tested against available monitoring data on chemical concentrations in edible aquatic species and concentrations in serum of adult men in Venice area to assess the reliability and applicability of the proposed tool to real complex scenarios. Full chain exposure assessment is then complemented by uncertainty and sensitivity analysis including local sensitivity methods, screening methods (e.g. Morris method), global regression methods (e.g. Standardised Regression Coefficients), and global variance based methods (e.g. FAST, EFAST, Sobol). These methods allow to follow for instance WHO (2008) recommendations to perform three stage uncertainty/sensitivity analysis, adopting qualitative, semi-qualitative, and quantitative methods. Integration of environmental and human exposure models in MERLIN-Expo allows comprehensive assessment of exposure and thus better characterisation of overall risk to human and environment especially in the case of higher tier assessment. This makes the tool interesting and promising for potential applications in different regulatory domains.

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Quantifying the Near-Field Exposures to Chemicals in Flooring Materials during the Use Phase

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There is growing evidence that near-field chemical intakes during use phase could be as important as environmentally mediated exposures. Thus, it is crucial to consider the exposures during the use phase when assessing the potential human exposures from consumer products. Chemicals encapsulated in products can be a major emission source in the indoor environment and in the use phase, of which typical examples are chemicals in flooring materials. These chemicals can be released to indoor air leading to inhalation exposures, and can also migrate to the flooring surface and lead to dermal exposure via physical contact. The present study thus aims to characterize the near-field exposures from chemicals encapsulated in flooring materials. A parsimonious model which describes the diffusive emissions of chemicals from materials and the subsequent loss by ventilation was employed. This model can predict the fraction of chemical mass emitted to air and the chemical concentration on product surface using two exponentials. The predicted air emissions and surface concentrations are then multiplied by an indoor inhalation intake factor and a skin permeability coefficient, respectively, to calculate the product intake fractions (PiFs), which determine the fraction of a chemical in a product that is taken up by humans during its use phase. The exposure doses can then be obtained by combining the PiFs and the chemical content in the product. Chemicals studied include 13 chemicals (5 SVOCs and 8 VOCs) in flooring that have ToxCast Oral Equivalency Dose (OED) data. PiFs were calculated for a child under 5-yr old in a

typical North American household with 3mm-thick flooring. Two extreme times points were chosen: 50 days and 15 years. All compounds have inhalation intake dominating except Bisphenol-A, whose dermal intake dominates. VOCs generally have higher exposure doses than SVOCs. Average daily dose ranges from 10^{-4} to 10^{1} for the first 50 days of flooring use and from 10^{-4} to 10^{-1} for 15 years. The exposure doses for most chemicals over 15y years are below OEDs, while half chemicals have doses higher than OEDs during the first 50 days, demonstrating the importance of the time scale when assessing exposure doses. This study shows that chemicals in flooring materials can lead to significant near-field human exposures, especially during the initial period (first 50-100 days) of flooring use, and further investigation is warranted.

Life Cycle Data and Modeling Developments - From Data Collection to Usage (I)

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Towards a Global Network of Interoperable LCA Databases

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1. Introduction International LCA dialogue has been formalized between countries within The "International Forum on LCA cooperation" since 2011 in order to facilitate the implementation of Sustainable Consumption and Production policies. Within this dialogue, cooperation on and access to global data has been identified as a must in order to reduce the cost and increase applicability of LCA studies in a context of a global economy. Therefore, the countries, with the support of UNEP, launched the Global Network of Interoperable LCA databases (the "Global Network") and agreed on a shared ambition that by 2017 the LCA user has a global and easy access to the main LCA databases with assessment of "fitness for purpose". 2. Materials and methods The Steering Committee is the governance body of the initiative and it is supported by the working groups, who are the operational units, created for a limited duration and focused on particular tasks products and deliverables. Three working groups (WG) have been launched: i) "Network Architecture and Technology" ii) "Nomenclature and iii) "Metadata descriptors". Working groups are co-chairs by experts and gather more than 50 experts worldwide. Each working group has defined a workplan, timeline and corresponding budget for the duration of the project. 3. Results This presentation will provide the latest update following up the 2016 face-to face meeting, scheduled in Brasilia from 14 to 16 March 2016. As of now, the following deliverables are planned to be completed: **Network Architecture and Technology** Review of existing examples and technologies for interoperable databases Development of functional requirements of the interoperable network of LCA data First discussion on a list of viable network options **Nomenclature** Agreed elementary flows mapping file ready for review Critical review of existing nomenclature mapping systems **Metadata descriptors** Identification of data quality assessment needs, with a focus on meta-information **\n** 4. Conclusion In conclusion, enhanced data accessibility and interoperability will benefit the whole community and the mainstream applicability of LCA, and is the foundation for key sustainability initiatives. Policy makers are relying on it for e.g. developing sound SCP policies. Industries will be able to base their innovation and strategic sustainability decisions on more robust information.

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BONSAI recommendations and procedures for implementation of an open source database

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The BONSAI database (https://bonsai.uno) is an open source community initiative, using semantic web technology and data harvesting to increase the free access to structured LCI data. This presentation describes our recommendations and current work on implementing the BONSAI core database and system. We implement the core database as an RDF store. The RDf store is designed for storing supply-use data, defined as a flow-by activity matrix for the balanceable properties of flows between activities, further specified by location, time and macro-economic scenario (relevant for forecasted data). Each datapoint consists of a value together with an unit and an uncertainty estimate. Non balanceable flow-properties and metadata can also be stored with the supply-use framework. We recommend a separate matrix to store information on complementary and substitutability between products. Besides the core database, BONSAI will store algorithms and software code for estimating missing data, automatic data harvesting and accessing and data manipulation. From the supply-use data, the algorithms will allow the calculation of direct requirements tables and their Leontief inverse matrices in order to estimate the product footprints. The barrier for adding data to the BONSAI database should be kept as low as possible, and conflicting data sources allowed to co-exist. Instead, data quality should be scored and reviewed subsequently, allowing algorithms for quality-scoring and consolidation to provide tresholds for temporary inclusion/exclusion and to derive preferred datapoints.The integration of data at many different levels of detail requires algorithms for automatic disaggregation and gap-filling, using global product and activity breakdowns based on industry data sources and data from countries with detailed data. The first public beta-version should demonstrate proof-of-concept of the key aspects that differentiate BONSAI from other similar databases. This includes the modular approach to software design, the low barrier to data entry with subsequent open review, the completeness (integrating MRIO and detailed data), the natural language footprint query interface and the transparency of the underlying data and routines. This also implies that the data needs to be provided with a fully open license (CC-BY) that allows also commercial use.

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Coupling process simulation and life cycle assessment: applications to bio-based process investigation

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Simulation of manufacturing processes combined with environmental impact assessment gives engineers the opportunity to improve the environmental performances of the production system. Process simulation tools were used with LCA in order to study the influence of operating parameters and to estimate missing data for life cycle inventory. This work presents, through two illustration cases, the use of process software (Aspen Plus™) as a tool for inventory data estimation and for environment performance optimization. The first case illustrates the use of the software to estimate inventory data for the production process of H_2 from biogas reforming based on an anaerobic digestion plant inventory from the literature. The second example addresses an optimization of bioethanol steam reforming process to provide accurate conditions for sustainable H_2 production. A variation in process operating parameters was also performed to illustrate the environmental performance sensitivity and provide guidance as to research and development effort focus for the process improvement. The variation of the parameters was performed using two methods: intuitive approach, where the levels of all parameters are fixed except one varying, and a factorial Design of Experiments (DOE) method. The different process configurations were simulated in Aspen Plus™ to provide foreground inventory data for the LCA of H_2 production system. For both systems, H_2 from biogas reforming or from bioethanol, the substrate production greatly influences the environmental performances of the whole system, especially when the system comprises an end-of-life management scenario (first case). The second investigation (H_2 from bioethanol reforming) indicates that the increase of reforming temperature improves the environment performance of the H_2 production system. Whereas steam to carbon ratio has a marginal effect on the environment performance. The main outcome of this DOE method is the development of mathematical models that predict how changes in the reformer operating temperature and steam to carbon ratio affected the environmental performances. The systematic integration of simulation tools into the elaboration of environmental assessment of processes will bring scientific legitimacy to environmental evaluation by LCA. The simulation-based LCA can be very helpful to develop more sustainable processes and products. This axis should be further developed, for instance, by sharing the software databases.

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LCA Cloud: enhancing LCA data collaboration

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Data exchange is an important concern in LCA due to the high variety of existing data formats, nomenclatures, data quality criteria, etc. Some of the mentioned challenges have been already overcome in the free, open source LCA software openLCA by integrating the most complete import and export features, which include all the commonly used LCA data formats (i.e. ILCD, EcoSpold1, EcoSpold2, etc.), as well as by the harmonization of elementary flows for the different LCI databases available in openLCA Nexus. As a further step towards facilitating LCA data creation and exchange, a new web service providing cloud functionalities was developed and integrated into openLCA. This "LCA Cloud", which was developed as part of a project supported by the US Department of Agriculture (USDA), allows the upload and download of data sets into/from a data repository. Moreover, a merge tool was also implemented in openLCA to identify and solve conflicting changes in the data sets retrieved from the server. The RESTful web service for storing and providing LCA data in a uniform way was developed as a standalone web application. The JSON ld format is used for the exchange of data between the service and the openLCA software. This format is recommended by W3C for linked data and it can directly be parsed as RDF triples and, therefore, be directly linked to ontologies. Moreover, the web service contains also other functionalities such as querying of data sets by user defined criteria, validity check to avoid corrupting databases, user and repository management systems and copyright protection procedures. Additionally to the web service, a merge tool was developed and integrated into openLCA in order to identify and manage conflicting changes to the same dataset. This functionality is specially useful when several users work in paralell in the same data. A text comparision between the two versions of the dataset is done for each of the fields included in it (e.g. descriptions, locations, amounts, units, etc.). The differences found are then displayed highlighted in the tool's editor and the user has the possibility of accepting all, part or none of the modifications addressed. "LCA Cloud" and the merge tool are powerful features for data sharing using the LCA software openLCA, which enable not only loading and retriving data from web repositories managed by the users or by external hosts but also the identification of differences within several versions of the same data set.

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SuBoot - Sustainability Bootstrap Project
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Oftentimes individual data collection for a case is considered as one of the more time consuming stages of a life cycle assessment, while on the other hand data is available but needs to be processed from all kinds of repositories. A novel approach to rapidly create a reliable and sound data base is tested within the SuBoot project, which is built around three key ideas. (i) The request from consumers to consider sound, reasonable information about the sustainability of products, at point of sale; (ii) .Opportunities for businesses to increase their revenues and market shares if they focus on green and ethically produced goods; Inclusion of alternative sources for sustainability information, which are "untapped" today. The aim of SuBoot is, therefore, to be a system to massively collect life cycle and sustainability data from primary sources, and make it available in supply chains and to end users: empowering consumers by providing life cycle sustainability information at the point of sale. These aspects are integrated in a smart and smooth software system, including one or several data nodes, an app for end consumers, and a B2B application. The system will be presented for a practical case to highlight challenges and solutions.

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Water supply mix: spatial, temporal and user variability at European scale
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Usually, the water used by a particular user (domestic, industrial, agriculture, etc) does not come from a single source but rather from a mix of local and sometimes imported water sources. Depending on the origin of the water abstracted (river, lake, aquifer, sea, etc), the location (water abundant or poor), the volume being extracted and the season of the year (wet/dry), the resulting environmental impacts will differ. It is therefore very important that Life Cycle Inventory (LCI) databases contain information on these aspects for proper subsequent impact assessment. However, current LCI databases neither differentiate (sub-)watersheds nor include temporal aspects, as required by ISO 14046 for a proper water footprint and impact assessment. In addition, the differentiation between surface and groundwater and the consideration of unconventional sources such as increasingly used desalinated or reused water remains limited. Furthermore, information on the combination of water sources supplied to a specific user is very scarce, while for background processes the actual origin of water is usually unknown. The aim of this work is to develop a spatially and temporally specific water supply mix for different water user needs. As a starting point, the geographical scope is Europe. The water mix builds on 1) the well established concept of the electricity production mix in Life Cycle Assessment (LCA), 2) specific literature on inclusion of water in the LCI and the Quebec tap water supply mix, already integrated in ecoinvent v3.1. The water mix proposed includes: water origins, water users, spatial and temporal specification and technologies (water abstraction, water production and water distribution). Data collection on water abstraction was done at national and regional (i.e., watersheds) levels per European country. A great spatial variability of water origins for different water users has been observed. There is also a temporal variation throughout the year that should be considered. The water supply mix, a mix of water sources and related technologies to meet a user need at a specific time (season) and location (sub-watershed), provides reliable water use profiles for background (and foreground) systems in LCA and will allow for LCIA methods to distinguish between the trade-offs of the various impact profiles of a given local mix. This project will enable routine assessment of water-use impacts just as straightforward as the use of the electricity supply mix.

Improving the usability of ecotoxicology in regulatory decision-making: findings from a SETAC Pellston® Workshop

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How to improve the quality of ecotoxicity studies
J.W. Green, DuPont / Applied Statistics Group
This presentation will address how we can go about 'improving the quality and reporting of ecotoxicology studies'. By advancing these two aspects, we should be able to increase the number of studies that can be used in the risk assessment process. Overall, there is consensus around what constitutes a well-conducted ecotoxicity study; and that these factors should be required for publication in the peer-reviewed literature. These include, but are not limited to, confirmed exposures, minimum replication, appropriate controls and control performance, and data transparency and sharing. Implementation of these recommendations by journals, and adoption of these practices by researchers at the study performance level, will facilitate the evaluation of the quality of work by all scientists, and draw more defensible data into the regulatory decision-making.

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The student perspective
M. Agerstrand, Stockholm University / Environmental Science and Analytical Chemistry

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Panel discussion
M. Agerstrand, Stockholm University / Environmental Science and Analytical Chemistry
Moderated panel discussion focusing on questions that the panel members get in advance. Panel members: Allen Burton (University of Michigan, USA/editor ET&C), Thomas-Benjamin Seiler (Aachen University, Germany/chair of SETAC Europe Advisory Group on Science and Risk Communication), Natalie Burden (NC3Rs, UK), Henrik Sundberg (Swedish Chemicals Agency, Sweden) Discussion points: 1) Comments on the results from the SETAC Pellston workshop. 2) How can scientific journals enhance and maintain the quality of ecotoxicity studies? 3) Do scientific journals have any responsibility to publish standard guideline studies? 4) What role do the institutions that fund research play? 5) How can SETAC contribute?

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Questions

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Evaluating reliability and relevance of ecotoxicity studies
C. Moermond, RIVM / Centre for Safety of Substances and Products
This presentation will address the following questions: What types of problems can be anticipated when evaluating ecotoxicity studies for regulatory risk assessment? What are the appropriate reliability and relevance evaluation criteria for peer-review publications? Can the available evaluation methods be used for regulatory risk assessment? e.g., Klimisch, CRED, EPA Soils (EcoSSLs), or other guidelines for evaluating ecotoxicology data, and methods of other disciplines – toxicology, evidence-based medicine, etc. It also will highlight frameworks that can be used to better design studies so they can be used in the regulatory process and what is needed for these studies to be evaluated for reliability and relevance to risk assessment and risk management.

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Weight of evidence evaluation
T. Hall, Bayer CropScience / Environmental Toxicology and Risk Assessment
In order for sound risk assessment and risk management decisions to be made, the Weight of Evidence (WoE) procedures must be objective, systematic and transparent. We reviewed the current quantitative and qualitative methods used by regulatory agencies and those found in the general literature. The goal was to develop a method that allow the weighing of the lines of evidence in a prospective risk assessment that then demonstrate the reliability, relevance, adequacy and consistency of the information. The general obstacles and difficulties when performing WoE evaluations in regulatory risk assessment were discussed. Through this evaluation a series of recommendations and approaches have been developed that allow for all scientific evidence relevant to a hypothesis to be considered and evaluates the strengths and value of the ecotoxicology data in the risk assessment.

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Panel discussion
M. Agerstrand, Stockholm University / Environmental Science and Analytical Chemistry
Moderated panel discussion focusing on questions that the panel members get in advance. Panel members: Anu Kapanen (ECHA, Finland), Jose Tarazona (EFSA, Italy), Jason Snape (AstraZeneca, UK) Discussion points: 1) Comments on the results from the SETAC Pellston workshop. 2) What is needed from researchers to

increase the use of peer-reviewed studies in regulatory risk assessments? 3) What can regulatory agencies and industry do to ensure that all relevant and reliable data is included in dossiers? 4) What are the barriers that exist, and potential solutions, to making industry-sponsored studies more transparent? 5) Future needs? 6) How can SETAC contribute?

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Questions

Contaminants of Emerging Concern in the Environment and their Management (II)

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Transformation products found in full-scale wastewater ozonation and biological post-treatment
C. McArdell, Eawag / Department of Environmental Chemistry; M. Boehler, Eawag / Department of Process Engineering; E. Borowska, M. Bourgin, Eawag / Environmental Chemistry; J. Fleiner, Eawag / Process Engineering; J. Hollender, Eawag / Environmental Chemistry; C. Kienle; R. Teichler, Eawag / Environmental Chemistry; H. Siegrist, Eawag / Process Engineering; U. von Gunten, Eawag / Water Resources
In Switzerland, a new water protection act was approved in spring 2014 which asks for the upgrade of around 100 out of 700 wastewater treatment facilities with advanced treatment to eliminate micropollutants within the next twenty years to enhance surface water quality. The WWTP Neugut in Dübendorf is the first facility in Switzerland where ozonation has been installed before sand filtration for the abatement of micropollutants. As the contaminants are not fully mineralized during ozonation, transformation products are produced. The elucidation of these transformation products and their potential adverse effects for the environment is of concern. In the scope of the study was: (i) determination of reactivity of compounds with ozone and identification of ozonation products (OPs) of selected compounds, (ii) investigation of the formation of OPs in the ozonation at the full scale facility in the WWTP Neugut and of their fate in different biological post-treatments, and (iii) investigations of different effects with bioassays in the laboratory and directly on the WWTP in a flow-through systems. In the study pharmaceuticals which occur in significant concentrations in WWTP effluents were investigated in detail. Species-specific second-order rate constants for the reactions of the molecular and dissociated forms of eight compounds with ozone were determined. The investigated compounds were found to represent a wide range of reactivity. For three compounds, different OPs were proposed from measurements with high-resolution mass spectrometry, MS2 spectra analysis and expert knowledge on ozone reaction mechanisms. Several OPs could be confirmed with available standards. N-oxides were quantified with high yields as the primary OPs for tertiary amines. In the bacteria bioluminescence assay performed on ozonated and non-ozonated samples of the three compounds cetirizine, fexofenadine, and hydrochlorothiazide, the toxicity was slightly increased during the ozonation of cetirizine, however, only at cetirizine concentrations higher than what is found in wastewater treatment. The assessments of the ozonation effluent of WWTP Neugut confirmed the results from lab studies concerning reactivity with ozone and the formation of OPs. Several OPs from the literature were also found after ozonation at this plant. The evaluated OPs were mostly stable in the different biological post-treatments investigated after ozonation.

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BIOMONITORING AND SOURCE TRACKING OF DIOXINS IN THE NETHERLANDS
A. Arkenbout, Toxicowatch Foundation
In the region of Harlingen, situated in the north of the Netherlands, people are becoming very concerned about adverse health effects related to the possible emission of dioxins and other POPs by a waste incinerator installed in 2011. A monitoring programme using backyard chicken eggs, shows a serious pollution with dioxins and dl-PCBs in the environment of Harlingen harbour. In order to find the source of this pollution, the preliminary results of analysis of a long-term sampling of the flue gas of the incinerator will be discussed in relation to the congeners found in the backyard chicken eggs.

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Fate, metabolism and transformation of wastewater-borne pharmaceuticals and their transformation products in the aquatic environment and fish in European rivers
S. Perez, IDAEA-CSIC / Environmental Chemistry; J. Aceña, M. Hannemann, J. Peña, IDAEA-CSIC; D. Barcelo, IIQAB-CSIC / Dept Environmental Chemistry
The presence of pharmaceuticals residues in the aquatic environment has caused concerns about potential adverse effects on exposed wildlife. Pharmaceuticals prior to entering WWTPs have already been metabolized in humans which leads to biotransformation products with different chemical structures and physico-chemical properties compared to their parent compounds. After their excretion in conjunction with their human metabolites and their discharge into the sewer, they enter in WWTPs where they can be further transformed by secondary treatments and advanced treatments generating transformation products (TPs). Several studies have been reported the occurrence of pharmaceuticals, their human metabolites and their TPs in wastewater treatment plants (WWTPs), surface waters and different fish tissues, but still very little is known about their fate in fish. Fish have been shown to possess hepatic detoxification systems capable of metabolizing xenobiotics taken up from polluted waters. In this context, we propose to screen surface water samples and fish for a large number of TPs formed in WWTPs and rivers. In order to have a broad perspective of the transformation of pharmaceuticals detected in surface waters and their metabolism in freshwater fish a suspect screening of pharmaceuticals, TPs and their predicted metabolites was applied in all samples from around Europe. With this approach several pharmaceuticals and TPs, more than 50 compounds, were detected in surface waters. In fish more than 15 phase I metabolites were detected, corresponding to different hydroxylation reactions, and phase II metabolites such as glucuronide and taurine conjugates were tentatively identified. The suspect analysis of muscle allowed the detection of more than ten different pharmaceuticals. The analysis of fish bile has allowed for the detection of several phase I and phase II metabolites and suggests that the occurrence of drug metabolites in bile can be used as a surrogate for exposure of fish to pharmaceuticals. Currently, the identification of several TPs and metabolites still requires confirmation. In accordance with the literature, anti-inflammatories, antibiotics and psycho-active drugs have been the most commonly detected drugs. Thus this approach highlights that UPLC–HRMS is a powerful tool for simultaneous quantitative and qualitative analysis, allowing the search for suspected compounds, TPs and metabolites, their identification and the quantitation of target compounds.

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Removal of contaminants of emerging concern (CECs) during primary, secondary and tertiary wastewater treatment steps
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Contaminants of emerging concern (CECs), including pharmaceuticals and hormones, are secreted by humans and end up in wastewater treatments plants (WWTPs) that are not designed to treat them, resulting in the occurrence of CECs in water bodies.This study consists of both experimental and modelling work and aims at understanding the removal of 19 target CECs, including hormones, pharmaceuticals and some of their main metabolites, during primary, secondary and tertiary treatment. The WWTP studied is the Guelph WWTP, ON, Canada (120,000 PE). The treatment train consists of primary clarification, activated sludge, rotating biological contactors and sand filtration. Sampling campaigns were carried out on four consecutive days in the summer, where 24-hr composite aqueous and sludge samples were collected. Aqueous and sludge samples were extracted by solid phase extraction and accelerated solvent extraction, respectively, and the extracted samples were analyzed by LC-HRMS. Conductivity was measured throughout the plant and used as a tracer for the calibration of the hydraulic model of the treatment plant that was built using the wastewater simulation software WEST (MikeByDHI.com). The hydraulic model was used to obtain load fractions, which were used along with experimental CECs concentration data to obtain reliable removal levels of CECs. Results showed that for some CECs, such as carbamazepine and codeine, the average mass loading in the effluent was as high as 20 g/d. It was also found that the majority of CECs had low removal rates in the primary clarifier stage and most of their removal took place in the activated sludge unit, with removal rates >70% for some of the target CECs. Concentrations of CECs in primary sludge ranged from 2 to 200 ng/g. The removal of CECs in rotating biological contactors, for which limited data is available in literature, and sand filtration was found to be < 30% for most of the CECs, although a few of them were efficiently removed by sand filtration. The experimental removal rates were used to calibrate a model that predicts the fate of CECs during primary and secondary treatment. This model can be used as a design and optimization tool in WWTPs.

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First Swiss wastewater treatment plant with full-scale ozonation - Ecotoxicological assessment of ozonation and several post-treatments
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Analysis ITAS; I. Werner, Swiss Centre for Applied Ecotoxicology / Department of Anatomy Physiology and Cell Biology
Emerging pollutants in wastewater, such as pharmaceuticals and personal care products, can be eliminated by advanced wastewater treatment techniques. Thus the discharge of these substances into freshwater ecosystems can be diminished and drinking water resources can be protected. Previous studies have shown ozonation to be a promising technique to eliminate micropollutants, even though it can produce reactive and potentially toxic transformation products. To remove these transformation products, which are often biologically degradable or bind to particles, post-treatments like sand filtration or granulated activated carbon are recommended by the Swiss Federal Office for the Environment (FOEN). The wastewater treatment plant (WWTP) Neugut is the first in Switzerland to apply a full-scale advanced treatment of wastewater with ozone followed by sand filtration. In the present study, the efficiency of ozonation and a number of post treatments to reduce ecotoxicological effects of biologically treated wastewater were assessed using a set of bioassays. Post-treatment methods were: a full-scale sand filtration, pilot-scale granulated activated carbon (GAC), and pilot-scale fixed and turbulent fluidized beds. *In vitro* bioassays were applied in the laboratory and *in vivo* bioassays with living organisms were performed in the laboratory and on site The investigations revealed that the wastewater treatment with ozone resulted in significantly reduced ecotoxicological effects, improved growth and photosynthesis of green algae, decreased toxicity to luminescent bacteria, and decreased estrogenicity, compared to effects measured in the waste water from the secondary clarifier. The post-treatments partially lead to a further decrease of the effects. Only the reproduction of water flea and oligochaetes was not influenced by any of the treated wastewaters compared to unpolluted control water, whereas fish partially showed developmental and histopathological effects after ozonation, which could be reduced with suitable post-treatments. The applied *in vitro* and *in vivo* bioassays showed that biological effects related to effluents can significantly be reduced by ozonation followed by a suitable biological post-treatment such as sand filtration or granulated activated carbon treatment. The study was financed by the FOEN (ReTREAT project) and by the EU in frame of FP7 (DEMEAU project no. 308339).

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Electrochemical oxidation of fluoroquinolone antibiotics: Mechanism, residual antibacterial activity and ecotoxicity

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Introduction Fluoroquinolone antibiotics have been detected in WWTPs effluents, surface water and various environment matrixes. Continuous release of FQs may induce antibiotic resistance and ecotoxicity to aquatic organisms and humans. Increasing studies have focused on the removal of FQs by Electrochemical advance oxidation processes (EAOPs). However, a comprehensive understanding of oxidation mechanisms of FQs during EAOPs has not been reported before. Furthermore, various intermediates and products of FQs might form during EAOPs and release into the environment. Little attention has been paid to evaluate the environmental effects of electrochemical oxidation effluents which contain oxidation products with potential hazard. The objective of this paper is to comprehensively study electrochemical oxidation mechanisms of ciprofloxacin (CPF), norfloxacin (NOR) and ofloxacin (OFL) and evaluate potential environmental risks of FQs and their oxidation products. **Methods** A commercial flow-through EC cell was respectively connected to a QTRAP 2000 and a high-resolution ESI-FTICR-MS Ultra. Scaling up reactions were conducted in a mg level EC cell. The disk diffusion test indicates antibacterial activity was carried out according to Disk Diffusion Test Methodology by EUCAST with slightly modification. The algal growth inhibition tests with *Desmodesmus subspicatus* were following to OECD 201. The EC_{50} were calculated by software ToxRat Professional XT. **Results** Eight oxidation products were respectively elucidated for each parent compound. Oxidation pathways were proposed on basis of product structraures and transformation trends. Oxidation and ring cleavage of the piperazyl substitute represent the main oxidation mechanisms of FQs by electrochemical oxidation while fluoroquinolone core remained unchanged. CPF, NOR and OFL respectively formed larger inhibition zones than their oxidation product mixtures at all exposure concentrations. The results reveal that the oxidation products of CPF, NOR and OFL collectively exerted weaker activity than their parent compounds after electrochemical oxidation. The EC_{50} values of CPs and NPs are almost the same as that of their parent compounds respectively whereas the EC_{50} value of OPs are much lower than that of OFL after 72h exposure. Our results indicate toxic intermediates were generated during electrochemical oxidation which contributed to the combined toxicity of oxidation mixtures to the green algae.

Flame Retardants: Alternatives, Environmental Fate and Toxicity (I)

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Legacy and emerging Flame Retardants in the Global Ocean Atmosphere

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The use of flame retardants (FRs) is a common practice to avoid the flammability of consumer goods like textiles, furniture, foams, electronics and building materials. FRs exhibit environmental persistence and long range transport potential, behaving like persistent organic pollutants (POPs). Due to their global occurrence, bioaccumulation potential and toxicity some legacy FRs are regulated (e.g. poly brominated diphenyl ethers (PBDEs)) and thus, alternative FRs have been launched in the recent years to substitute the banned compounds (e.g. dechlorane plus (DP) and organophosphate esters (OPEs)). This study includes the occurrence of 15 PBDEs, syn- and anti-DP isomers and 2 dechlorinated forms of anti-DP, and 14 OPEs analyzed and quantified in oceanic aerosol. Over 110 air samples were taken during the Malaspina circumnavigation cruise between 2010-2011 which crossed the north and south basins of Atlantic and Pacific oceans as well as the Indian Ocean, between 30ºN and 40ºS covering all the tropical and subtropical oceanic gyres. Moreover, dry deposition fluxes were calculated with a parametrization of the deposition velocities for each FR. This is the first combined assessment of legacy and emerging BFRs, including PBDEs, DPs and OPEs in the oceanic atmosphere, showing the ubiquitous presence of these pollutants even in remote oceanic regions, and the atmospheric load to the open ocean through aerosol dry deposition.

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Emerging Flame Retardants in Air in the Canadian Great Lakes Basin

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Persistent organic pollutants (POPs) and other priority organic pollutants are being measured in air in the Canadian Great Lakes Basin (GLB) at land-based stations since 1990, in support of the Canada/US Great Lakes Water Quality Agreement (GLWQA). Air samples were screened for new chemicals of concern, such as emerging flame retardants (FRs), to assess the risks that these pollutants may pose to wildlife and humans in the GLB. In this study, results from atmospheric measurements of emerging brominated (BFRs) and chlorinated (CFRs) flame retardants are reported in comparison with measurements of polybrominated diphenyl ethers (PBDEs). Hexabromobenzene (HBB), allyl 2,4,6-tribromophenyl ether (ATE), 1,3,5-tribromobenzene (TBBz), anti- and syn-dechlorane plus (anti-DP and syn-DP) were dominant FRs found at two GLB sites, namely Point Petre on Lake Ontario and Burnt Island on Lake Huron, with levels mostly lower than PBDEs. Similar to the PBDEs, lighter FRs in air showed a strong sesaonality with higher concentrations in the summer months. Higher FR air concentrations were found at Point Petre, which is closer to urban developments, than at Burnt Island. While air concentrations of PBDEs were declining in Great Lakes air between 2005 and 2013, which may be related to control efforts, no consistent changes in air concentrations were observed for most emerging FRs (2009-2013).

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Organophosphate Flame Retardants in ambient air of Bursa, Turkey

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Flame retardants are chemicals that are added to many consumer products such as plastics, electronics, textiles, and foams for furniture, automobile interiors and many others. The most widely used flame retardants are polybrominated diphenyl ethers (PBDEs), although others have been used, including hexabromocyclododecane (HBCD), tetrabromobisphenol-A (TBBP-A), organophosphate flame retardants (OPFRs), decadibromodiphenyl ethane (DBDPE), 1, 2-bis (2,4,6-tribromophenoxy)ethane (BTBPE) and others. Over the recent past, PBDEs have undergone much scrutiny because of their persistence and toxicity. Research interest has started to focus on the non-PBDE flame retardants. Although organophosphate flame retardants (OPFRs) have been widely used for decades, after some studies in the 1980s indicated that these chemicals degrade quickly in the environment no more studies were carried out on them for a long time. More recently, a few studies have indicated that they are persistent and are subject to long-range transport. There is now increased interest in understanding their environmental fate and transport. In Turkey, there are few studies on PBDEs in the environment, in indoor air and dust however, to the best knowledge of the authors there are not any studies on the organophosphate flame retardants in ambient air in Turkey. Levels of flame retardants in indoor air and dust were found to be at ng levels indicating that such chemicals are abundant in Turkish environment. However, detailed surveys were not conducted to investigate such chemicals in Turkey. This highlights the need to understand the

levels, sources, and movement of flame retardants in the Turkish environment. In this study, re report levels of certain OPFRs in ambient of Bursa, Turkey that was obtained during the course of a 1-year passive sampling study.

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Monitoring levels of legacy and emerging flame retardants in indoor environments: a multi-location study between Norway and the UK

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Living in the post-PBDE era entails production, commercial use and gradual replacement of fire-inhibiting compounds with newly manufactured chemicals also known as "emerging" halogenated flame retardants, such as EH-TBB & BEH-TEBP (Penta-BDE replacement), BTBPE (Octa-BDE replacement), DBDPE (Deca-BDE replacement), Dechloranes (DPs) (Deca-BDE replacement) or organophosphorus flame retardants (PFRs; TPHP, TCPP etc.). The main objectives of the present study are: a) To assess the occurrence of legacy and emerging alternative FRs across two European countries (the UK and Norway) with respect to human exposure via dust ingestion. No data have been reported yet on emerging PFRs, also known as novel PFRs (NPFRs), in dust from residential or occupational microenvironments neither for the UK nor for Norway. In addition, this is the first study reporting significant levels of NPFRs including isodecyldiphenyl phosphate (iDPP) and trixylenyl phosphate (TXP) in indoor microenvironments. b) To identify possible FR sources with respect to the collection source and country of origin, taking into consideration source variability and its impact on usage of emerging FRs in consumer products. Dust samples (n=22) from vacuum cleaner bags (10 houses and 12 stores, offices, libraries) in the UK were collected during August 2013 – December 2013. Dust samples (n=10) were collected from vacuum cleaner bags (houses) in Norway during November 2013 – April 2014 as a part of the A-TEAM cohort sampling within the framework of the '*Advanced Tools for Exposure Assessment and Biomonitoring'* (A-TEAM) project, a Marie Curie Initial Training Network aiming to establish tools for human exposure biomonitoring of emerging FRs. In the present study, a broad range of legacy and emerging FRs were studied, including PBDEs, novel BFRs, PFRs and novel PFRs. A commericial shift and gradual replacement from the phased-out FRs to emerging FRs is evident. We are the first to identify and report on NPFRs, such as iDPP and TXP in indoor dust originating from residential and occupational environments, suggesting extensive usage of NPFR-coated consumer products in the UK and Norway. This project is financially supported by the European Commission FP7 Marie Curie Initial Training Network "A-TEAM" grant number 316665.

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Biomagnification study of hexabromocyclododecane isomers in freshwater fish and invertebrates

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Since 2011, hexabromocyclododecane (HBCD) is subject to authorization (Regulation N° 143/2011 EU) and considered as persistent, bioaccumulative and toxic (PBT); this compound is included in Annex A of the Stockholm Convention since 2014, and became a priority substance for water in Europe since 2013 (Directive 2013/39 / EU). As for several other priority substances, the Environmental Quality Standard (EQS) for HBCD is set for biota (i.e. fish), meaning that compliance checking will have to refer to fish. HBCD is an additive brominated flame retardant (BFRs), used especially in the building sector, thermal insulation, textiles, electrical equipment. In this context, the aim of this work was to determine trophic magnification factors (TMF) in various rivers and streams in France, and to examine their spatial-temporal variability. HBCD isomers (α, β and γ) were analysed in fish and benthic invertebrate tissues. These samples were collected from five study sites representing different river conditions (catchment size and anthropogenic pressure). All the concentrations found in fish and invertebrates were below the Environmental Quality Standard EQS_{biota} (167 $ng.g^{-1}$ wet weight (ww)). α-HBCD displayed the highest trophic magnification factor and significant differences were observed between sites. The determination of TMFs by 3 different models (linear regression, censored regression and GLMM) has shown the importance of the regression model used for TMF assessment. It is therefore important to consider the different aspects of data treatment (e.g. heterogeneity in the number of samples), all the while knowing that each model has its limitations.

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Fishing of unknown halogenated environmental contaminants in environmental sentinel based on isotopic pattern and mass defect through untargeted high resolution mass spectrometry profiling

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Bioaccumulation of Persistent Organic Pollutants (POPs) is predictably associated with the compounds characterised by high lipid solubility thus an ability to accumulate in the fatty tissues of living organisms for long periods of time. POPs increase in concentration in the food chain and are typically halogenated organic compounds. In the case of known substances, targeted approaches mainly based on mass spectrometry (MS) are available to characterise human exposure (environment, food) as well as body burden (human tissues and fluids). These quantitative methods do not permit however to detect non yet described exposure markers, i.e. degradation products of known substances or unknown/emerging substances. Last generations of high resolution mass spectrometers (HRMS, e.g. orbitrap or TOF) open the way to untargeted full scan footprints as a new methodological approach for addressing this issue. Large datasets arise from such HRMS couplings with chromatography from which the extraction of the useful information stands as a central question, thus requiring the development of fit-for-purpose intelligent data processing, analysis and visualisation tools, supported by appropriate biocomputering solutions. In our work, we discuss the proof of concept of a new methodology capable of seeking unknown organohalogenated compounds in environmental sentinels. Our strategy relies on an automated peak picking step based upon the centWave function (xcms package, R environment), a VBA script developed for pairing features according to the exact mass difference between Cl and Br isotopes to filtering potential organohalogenated clusters among full scan HRMS datasets. H/Cl-scale mass defect plots were used to visualize the datasets before and after filtering. The filtering script was successfully applied to an LC-HRMS untargeted profiling dataset generated from an eel sample muscle extract obtained after pressurised liquid extraction and a non selective liquid-liquid partitioning between concentrated sulphuric acid and hexane. From 9,789 initial features observed in the eel muscle extract, 1,994 features were paired in 589 clusters containing at least an M+2 contribution (indicating a Cl or a Br atom), allowing for realistic manual investigations of filtered clusters. Hexabromocyclododecane, chlorinated paraffin series and other candidates were fished and for some of them identified.

Microplastics in the environment: Sources, Fate and Effects (II)

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What, when and where? Assessing temporal and spatial trends in microlitter>10μm in coastal waters west of Sweden

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Comparable datasets are a luxury within marine litter research, due to the novelty of the field. These golden glimpses of information provide valuable insights in trends of abundance and characteristics, to understand sources and pathways and can also help us plan sampling and monitoring to achieve and increased accuracy in future comparisons. On the Swedish west coasts measurements of $\text{microlitter}_{>10\mu m}$ have been carried out at 14 locations using comparable methods at 4 occasions during 3 years.In a recently initiated project these datasets are evaluated to achieve a detailed overview of differences in composition and abundance. Preserved samples of commonly found anthropogenic microscopic litter such as blue, red and white particles and fibers are additionally being analyzed using FTIR. Initial comparisons of the datasets show that a consistently higher amount of particles were found on the smaller sized filters (10μm). Interestingly the composition here also differed, with a often higher amount of fibers and black particles on the smaller filters in relation to the classic polyolefin and expanded polystyrene microplastics on the filters with a wider pore size (300μm). The industrial harbor area by Galterön separates from the others on account of the relative high composition of synthetic fibers, red particles and black particles in the range of 50-100 μm. FTIR analysis are being carried out to further investigate these relationships, and to see how they might relate to activities in the area. The city areas of Älvsborgsbron, Skalkorgarna and Danafjord show some separation form the others, which is clearest in the results from 2013. The strongest separation is for Älvsborgsbron which is also the sampling point closest to the urban areas of Gothenburg which fits earlier observationswhere cities hav e been identified as a source of microlitter. The compositional differences here show a high amount of black particles with a probable origin in incineration processes, it also shows a high composition of plastic particles and to some extent also blue particles. The initial results of the ongoing analysis show higher amounts but also clearer trends in composition for the >10 μm samples than for the samples collected with a 300 μm net. It is therefore suggested that sampling and monitoring of smaller size classes of microlitter are important to understand temporal and spatial differences in microlitter abundance and composition.

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Neustonic microplastics in the Bay of Brest (Brittany, France): composition, abundance and spatial distribution

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Since a decade, microplastics represent an increasing concern for science and society with many studies establishing their fate and their impact in aquatic ecosystems. Many studies have been reported microplastic occurrence throughout the world ocean and in different environmental matrix such as water, sediment and biota. Some of these emergent contaminants can release toxic chemical compounds added during their manufacturing or adsorb from the surrounding seawater, both being possibly released in marine environment and biological matrices. In this context, studies aiming to determine the extent and impact of this pollution for marine ecosystems biodiversity and services are of great interest. In the present study, the abundance, composition and spatial distribution of microplastics were investigated in April 2014 from surfaces water of the Bay of Brest (Brittany, France), which is divided in three zones (north, central and south) characterized by different hydrodynamics and anthropogenic pressures. Microplastics particles were of the following classification: 0.3-1 mm, 1-2 mm and 2-5 mm. A combination of static image analysis of particles and automated Raman micro-spectroscopy was used to obtain the counting, size, shape and chemical composition of a large number of particles for each sample. 35 % of visually sorted particles were not identified as plastic polymer, so morphological criteria such as color, shape or texture are insufficient to assert that a particle is a plastic polymer. Eight of the nine stations contained microplastics particles and a mean concentration value of 0.269 particles / m^3 (i.e. 0.619 mg / m^3) was estimated. Only 65 % of particles collected at surface water were identified as plastic suggesting that visual sorting is not enough. A majority of polyethylene (76 %) was identified. The spatial distribution of microplastics in the Bay of Brest seems to be different between the three zones: the central zone exhibited the highest value (0.540 particles/m^3), followed by the North zone (0.218 particles/m^3), whereas only few microplastics were collected in the south part (0.049 particles/m^3). The south zone was mostly polluted by an important concentration of fibers but analyses are currently ongoing to identify their chemical composition and confirmed their plastic nature.

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Abundance of microplastics and adhered contaminants in the North Atlantic Ocean

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Marine plastic contamination has increased steadily over the last decades, and plastic debris account for more than 90% of the marine litter encountered by marine wildlife. The majority of the plastic litter can be characterized as microplastic (MP). Those plastic types that have a density below that of seawater will float on the sea surface for a prolonged period of time, and will therefore be transported with currents. This has led to a concentration of plastic debris in oceanic gyres. One of the five major oceanic gyres is situated in the North Atlantic Ocean and elevated concentrations of MPs have been reported in this area. Apart from direct physical effects such as clogging of gills and entanglement, plastic may also serve as a vector for other lipophilic contaminants, which can bind to and be transported with the plastic. The importance of such vector effects are not fully understood and are currently being discussed in the scientific literature. The current study reports findings and analyses of microplastic samples along a transect from the Caribbean Sea into the North Atlantic Gyre conducted in spring 2015. The aim was to: i) quantify abundance of MP ii), estimate relationships between size and distribution of MPs, iii) determine concentrations of lipophilic contaminants adhered to the MPs and iv) generate a GIS based map where data are included, that subsequently can be expanded to include data from other surveys and thus help inform decision makers about the marine plastic pollution. The studies showed that MPs dominated by PE and PP are abundant in surface waters of the North Atlantic Gyre and that smaller fractions were more dominant then larger. The analyses further documented that PCBs and PAHs can bind to the MPs at concentrations higher than those observed on biosamples. This illustrates that MPs can be important as vector for other contaminants. GIS mapping is a very useful communication tool that can be used to inform the public and policy makers, and thus facilitate measures to prevent future pollution. The GIS map is a useful tool for updating the data and displaying them for the scientific community as well as the public and policy makers. It may therefore be an effective tool in the work towards promoting mitigation measures.

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Microplastics in the Rhine-Main area in Germany: Occurrence, spatial distribution and sorption of organic contaminants

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Synthetic polymers are one of the most significant pollutants in the aquatic environment. Serious effects are expected from so-called microplastics (particle size The sediment was sampled from the river Rhine, the river Main and the stream Schwarzbach. Sampling was done by a randomized sampling approach. Small sediments samples along the shoreline of the rivers were taken and combined to batch samples for each sampling site. Using a modified density separation with subsequent sample clean-up and Fourier transform infrared spectroscopy or microscopy (FTIR), microplastics were separated from river shore sediments, resulting in large amounts of microplastic particles of up to 1 g kg^{-1} or up to 4000 plastic particles kg^{-1}. The identification by FTIR showed that polyethylene, polypropylene and polystyrene were the most abundant polymer types in the sediments, covering over 75% of all plastics identified. Furthermore, a possible transport of microplastics from tributaries to main streams was indicated by the detection of identical pellets at sampling sites in the River Rhine and in the Main mouth. Microplastic concentrations did not correlate with the population density of the sampling area. One sampling site was monitored over two years in intervals of five to nine months. Comparable concentrations were detected in the sediment and suggest a constant pollution of the river shore sediments with microplastics. Additionally, large microplastics (>500 μm) were extracted using ultrasonic-assisted extraction or extraction by dissolution and subsequent size exclusion chromatography. The extracts were analyzed by GC/MS and LC-MS/MS using target screening methods and non-target approaches. Different pesticides or polycyclic aromatic hydrocarbons were identified in the polymer particles, suggesting that microplastics can act as a sink for hydrophobic contaminants. Moreover, several plastic additives such as phthalates or chlorinated flame retardants were identified. For this reason, it is very likely that microplastics act as a direct source for these chemicals in aquatic systems. The results of this study stress the urgency for the mitigation of the plastic particles in the aquatic environment.

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Microplastics in inland waterways and coastal waters - origin, fate, and impact

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The accumulation of plastic debris in aquatic environments is one of the major but least studied human pressures on aquatic ecosystems. Under environmental conditions, larger plastic items degrade into smaller particles, so-called microplastics (MP) (< 5 mm in diameter). MP resulting from degradation processes are classified as secondary MP, while primary MP are produced as such for industrial purposes. MP particles represent freshwater contaminants of emerging concern. However, to assess the environmental risks associated with MP particles in freshwater ecosystems, comprehensive data on their abundance, fate, sources, and biological effects are needed. Therefore the research project "Microplastics in inland waterways and coastal waters – origin, fate, impact" by the Federal Institute of Hydrology (BfG) aims at establishing scientific principles for assessment, monitoring and regulation of MP in freshwater aquatic environments (water and sediment). Within this project the following tasks will be addressed: (1) occurrence (quality and quantity) of MP in inland waterways, (2) characterization of quantitative and qualitative input in waterways and coastal waters (mass balance study), (3) bioaccumulation potential of MP, (4) investigation of biological effects of MP on aquatic organisms, and (5) risk assessment for aquatic organisms and aquatic environments. On this account the research project consists of 5 work packages: (I) sample preparation and qualitative description, (II) development and validation of quantification methods, (III) biological effects, (IV) modeling and monitoring, and (V) leaching, impact, and risk assessment; of these the first results will be presented here.

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Microplastics in various compartments of the urban water cycle

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If the occurrence of microplastics, particles smaller than 5 mm, in both marine and continental environments have been studied, their inputs are still very poorly identified and only few works focused on their sources. This work aims at assessing the microplastic contamination in different compartments of the urban water cycle. Greywater and wastewater were first studied. Washing machine effluents have been collected in the houses of four volunteers. Three consecutive washes were considered, the first one with an empty washing machine, the second

one with the washing product only, and the last one as a regular wash. Microplastics in WWTP influents and effluents were also analyzed during 3 campaigns. Conventionnal 24-hour averaged samples were collected. Atmospheric fallout and rainwater were collected in two different sites, one urban (1 year) and one sub-urban (a half year), through a collection funnel. Stormwater samples were collected at the outlet of a small sub-urban catchment during six rain events. The two first samples of washing machine effluents (empty machine and without clothes) contained between 120 and 150 particles/L. When clothes are washed, concentrations between 8850 and 18700 particles/L were encountered, confirming the large contribution of the clothes. High levels of fibrous plastics are found in WWTP influents (260 – 320 particles/L) while the concentrations in the effluents are in the 14 – 50 particles/L range. A microplastic removal rate between 83 and 95% have been estimated. Throughout the year of monitoring (urban site), an average atmospheric fallout of 110 ± 96 particles/m^2/day (mean ± SD) was encountered. On the suburban site, a 6-month monitoring shows an atmospheric fallout around 53 ± 38 particles/m^2/day. Through all the monitoring period, a significant difference between the atmospheric fallout on the urban and the sub-urban site was found. By considering the rainwater volume collected on the atmospheric fallout funnel, a median concentration of 40 particles/L can be estimated. In runoff, the first results indicated that the microplastic concentrations lies in the same order of magnitude, i.e. between 39 and 60 particles/L range. To date, further work is required to evaluate the contribution of total atmospheric fallout to stormwater pollution.

Fate, Effects and Risk Assessment of Chemicals in Aquatic and Terrestrial plants (II)

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Ecosystem services approach to pesticide risk assessment and management of non-target terrestrial plants: recommendations from 2 SETAC Europe workshops

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The registration of Plant Protection Products (PPPs) in the EU falls under Regulation 1107/2009, which recommends a tiered approach to assessing the risk to non-target terrestrial plants (NTTPs). However, little information is provided on how to perform and implement higher tier studies or how to use them to refine the risk assessments. Therefore, two workshops (April 2014, Sept 2015) were organized with the aim of: developing a framework for a higher-tier approach to assessing the risk of PPPs to NTTPs; providing expert opinion and advice as input for the ongoing revision of the terrestrial ecotoxicology guidance document and NTTP risk assessment procedures. The recommendations agreed to by the first workshop relate to the three main themes, i.e. specific protection goals, risk assessment and mitigation. The participants of the workshop adopted the European Food Safety Authority (EFSA) approach of using an ecosystem services framework for identifying specific protection goals. First, delivery and protection of ecosystem services were discussed for in-crop, in-field *and* off-crop, and off-field areas. Second, lower and higher tier risk assessment methods, including modelling approaches, were evaluated and the benefits from these options were addressed. Third, options for risk mitigation of spray drift and run-off were discussed and evaluated. A number of concerns were raised during the workshop and literature reviews were performed and data collected in order to reduce uncertainty. These actions focussed on the protectiveness of standard test species for wild species; the protectiveness of regulatory endpoints for reproductive endpoints; the methods and endpoints for multispecies or field-studies; and the importance of different exposure routes for non-target terrestrial plants. The second workshop built upon the results of these literature reviews, the recommendations of the first workshop and the EFSA opinion on risk assessment of PPPs for NTTPs. The main charge questions identified for the second workshop were: how to address reproductive endpoints; how to mitigate risks; how to conduct higher tier tests. A higher-tier framework for NTTP risk assessment was proposed. Recommendations from both workshops will be presented.

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Sensitivity of NTTPs to plant protection products - vegetative and reproductive endpoints: Literature review and analysis for SETAC AG Plants

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A literature review was performed comparing vegetative and reproductive endpoints for non-target terrestrial plants (NTTPs) aiming to test the hypothesis that reproductive endpoints are more sensitive than the standard vegetative endpoints currently used to inform the pesticide risk assessment. In addition the EFSA Scientific Opinion [1] was considered, in which, among others, also reproductive endpoints were discussed to be more sensitive than the currently assessed vegetative endpoints. The dataset listed in [1] was assessed in parallel to the full data set (that also included confidential industry data). In addition current proposals to move from ER50 to ER10 endpoints were investigated. Although cases are reported of reproductive endpoints considerably lower than vegetative endpoints, based on the data assessed here the difference between the two was found to be minor, on average just a factor of ca. 1.5. Even if both endpoints (vegetative and reproductive) were always determined and the lowest of the two was used in the RA, the average gain in protectiveness would just be a factor of ca. 2 (based on the EFSA dataset). In contrast, the difference between ER50 and ER10 was greater than a factor of 6. A change of the effect level used in the RA (ER50 to ER10) would thus increase the conservatism of the tier1 RA for NTTPs considerably. The ecological relevance of a 10% reduction at the population and landscape level, advantages and disadvantages of the proposed change in effect level, a potential alternative by using the ER25 (or ER50 with an additional assessment factor), and if there is a need for an increase in conservatism of the tier1 risk assessment will be discussed.

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What information can we gain by including trait-based endpoints for plants in ecotoxicological tests? Case studies with Myriophyllum spicatum, the new OECD test organism for rooted aquatic plants

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In plant ecology, the trait concept provides functional markers for plant functions. Plant functional traits (morphological, physiological, phenological) reflect ecological strategies and determine how plants respond to environmental factors. Trait variability can reflect adaptations or acclimations at the intraspecific level. We hypothesize that morphological and physiological traits change in the presence of certain pollutants. Leaf, stem and root mass fractions (LMF, SMF, RMF), dry matter content (DMC) and stoichiometry, the content in carbon, nitrogen and phosphorus and resulting C:N:P molar ratios in plant tissue are traits that can provide information about effects at the individual level or allow extrapolations on ecological interactions and ecosystem processes. We tested our hypotheses exposing sediment-grown *Myriophyllum spicatum*, the new OECD test species to arsenic or mixed herbicide. Experiment 1 had a cross-factorial design with two CO_2-levels (low and high, LC and HC) and four arsenate levels. Experiment 2 exposed plants to isoproturon (IPU) or mesosulfuron-methyl (MSM) alone or in varying combinations. After two weeks, we measured final plant length, fresh and dry weight and dissected a part of the shoots into leaves, stems and roots, and determined C, N, and P contents. In experiment 1, arsenate and/or CO_2 availability affected morphological and physiological plant traits. SMF increased in arsenic-exposed plants, and was higher in the HC treatments. RMF declined to one third at high arsenic levels. LMF was affected both by CO_2 availability and arsenic levels. Arsenic level affected the DMC of leaves, stems and roots, but only roots exhibited a lower RDMC under LC treatments. Arsenic affected the N and P content and resulting C:N and C:P ratios in the whole plant, while CO_2 availability affected C content and CP and NP ratios. In contrast, we observed very little effects on C, N and P content in leaves. In experiment 2, IPU alone had no effect on LMF, but with increasing proportion of MSM, the LMF declined strongly. At the same time, SMF increased strongly with increasing MSM. With increasing MSM, the LDMC increased strongly compared to solvent controls or IPU. We conclude that incorporating different plant traits as endpoints with *M. spicatum* can provide valuable information useful to predict ecological effects of pollutants. Our experiments have shown that such changes in plant traits are frequent.

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Demonstration of Reciprocity of Effects of four Sulfonyureas on Myriphyllum spicatum

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The dependence of toxicity on not only the dose, but also time was first described by Haber. Haber's law states that the incidence and/or severity of a toxic effect depends on the toxic load i.e., the exposure concentration times the duration of exposure. For toxicants that block an enzyme's activity, the effect will depend on the number of bound molecules *per unit time*. Sulfonylureas have been known to follow this pattern of action. These herbicides act by inhibiting the ALS enzyme

responsible for producing branched amino acids. Given that growth is a time-rated process, large growth inhibitions exerted during short periods are comparable to lower inhibition over more prolonged periods. In this series of studies, we demonstrated that the inhibitory action of four sulfonyureas on the aquatic macrophyte *Myriophyllum spicatum* follows Haber's law. Two distinct exposure regimes (1- and 3-days), but at reciprocal exposure levels were run, meaning that exposures expressed on a TWA basis were the same. During each variable duration test, three termination intervals were conducted, at 3, 7 and 14 days. At each interval, a set of replicates was terminated and biological data collected. The response of *Myriophyllum spicatum* to these sulfonylureas clearly follows Haber's law, in that the effects resulting from different exposure levels, but of reciprocal duration, were not statistically different. Either 1- or 3-day exposures of *Myriophyllum spicatum* to the four SUs resulted in similar growth rate values for shoot length, and wet and dry shoot weights measured at either 3, 7, and 14 days after the beginning of exposures. There is previous data on metsulfuron methyl showing that EC_{50} values *Lemna minor* exposed for 42-d of continuous exposure were not similar to those calculated for 4- and 2-d, but reciprocal (TWA) exposures. Also, a study with metsulfuron methyl on *Myriophyllum spicatum* showed the same result when comparing the same 21-d TWA, but with different peak exposure concentrations and exposure periods (1, 3, 7, 14 or 21d). The data presented here further validate the conclusion by EFSA in relation to the validity of a TWA for methsulfuron methyl and also agrees with data collected for the better-studied *Lemna* spp. These results allow to recommend that the reciprocity assumption, and therefore allowance for the use of a 7-day TWA concentration, should be considered as a standard refinement for the risk evaluation of sulfonyurea herbicides.

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Effects of pulsed mixtures and recovery in Lemna minor based on POCIS field measurements.
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Pesticide contamination, in particular herbicidal loading, has been shown to negatively affect Luxembourgish river quality. Such pressure can have direct impacts on autotrophic species, and wider indirect impacts on the freshwater community through the degradation of primary production. The EC report on the toxicity and assessment of chemical mixtures indicated a method to determine the best approach for the evaluation of pesticide mixtures in the environment. However it is difficult to incorporate the variety and combination of compounds sold, used, and detected in surface waters into bioassays. Literature regarding pesticide mixture toxicity shows the validity of the 'concentration addition' (CA) concept for mixtures of compounds with similar modes of action, and the 'independent action' (IA) concept for mixtures of dissimilarly acting compounds. However a mismatch exists between real field exposure patterns and typical laboratory tests. This study attempts to bridge this gap by directly using spiked mixtures of pertinent compounds based on whole field extracts. Mixtures were applied in pulsed scenarios determined from passive sampling campaign results, in order to determine the toxicity of these real environmental mixtures to a representative autotroph. High risk compounds were selected based on sales, use, and historical detection data, in combination with toxicity values and compound characteristics. Herbicidal loading was determined using passive samplers (POCIS) over eight months, spanning two years and twelve representative streams in Luxembourg. LCMS analysis of these samples indicated the time-weighted average herbicide load over the exposure period. Seven day bioassays using the floating macrophyte *Lemna minor* were then performed to determine the toxicity of six high risk compounds and spiked artificial mixture based on whole field extracts. Bioassays were performed in pulsed scenarios mimicking exposures determined from POCIS results. Early results indicate metazachlor to be the most toxic of the selected compounds (EC_{50} 0.0106 mgL^{-1}). Further tests will clarify the toxicity of these compounds in mixtures and pulsed scenarios to the floating macrophyte *Lemna minor*. Presented here are the results from: (i) *L. minor* bioassays using both whole spiked mixture, and pulsed mixture scenarios (ii) use of CA and IA concepts to fit *L. minor* pulsed and spiked mixturebioassay results.

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Pesticide mixture toxicity to algae in agricultural streams - field and laboratory studies
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Contamination of surface waters with multiple chemical pesticides is well documented, but little is known about their concerted effects on aquatic communities. We collected water samples from four streams in small agricultural catchments in southern Sweden during a whole year. In addition to pesticide analyses, we used these water samples to run algal growth inhibition bioassays with *Pseudokirchneriella subcapitata*. In addition, we identified worst-case samples observed in long-term monitoring of pesticide water concentrations (> 80 pesticides) and used these to run tests with reconstituted pesticide mixtures in algal growth medium to specifically test their combined effects on algal growth. Both for bioassays with *in situ* water samples and mixture toxicity tests we used controls that consisted of standard growth medium. *In situ* water samples were spiked with nutrients to rule out confounding effects of nutrients. Long-term monitoring data showed more than 10 pesticides in 63% or more of the 902 collected water samples, with 90-percentiles ranging 21–29. The average number of pesticides in a single sample ranged 9-18, while the maximum was 41. These results stress the complexity of exposure by pesticides in agricultural streams. However, despite the multitude of pesticides in a sample, toxicity was frequently set by one or a few dominating compounds that contribute to more than 90% of the mixture's toxicity. $\sum TU_{algae}$ for long-term monitoring data exceeded 0.1 on 28 occasions, 70% of which occurred between May and July, i.e. the period when spraying is most intensive. Bioassays with *in situ* water samples showed a significant inhibition of algal growth in 49% of the 61 samples that were run during a whole year. On four occasions algae grew better in *in situ* water than in controls. There was no relationship between growth inhibition and estimates of pesticide toxicity (i.e. PTI and ΣTU_{algae}), suggesting that also chemical stressors other than pesticides could have contributed to the observed inhibitions. Tests with reconstituted medium showed that significant inhibition of algal growth occurred in the range of 1–10x the $\sum TU_{algae}$ observed in monitoring data. For all mixtures but two, calculated EC_{50}-values were between 3 and almost 10-fold higher than literature values for the dominant pesticide. The calculated EC_{50}-values for most of the mixtures were 2–17 times higher than those estimated by the model of concentration addition.

Metals in the Environment: Fate, Speciation and Bioavailability in Water, Soil and Sediment (II)

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Investigating fractionation and speciation of metal(loid)s in sediment pore water samples
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In aquatic environments the fate and behaviour of inorganic and organic substances is often driven by biogeochemical gradients at the sediment water interface (SWI), like gradients of e.g., the oxygen concentration, the redox potential or the pH value. Important transport processes at the SWI are sedimentation and resuspension of particulate matter and diffusional fluxes of dissolved materials. Beside this, the speciation of elements at the SWI is important to asses relevant reactions as well as potential effects. To investigate the factors and processes that govern the distribution and fluxes of nutrients, metals and metalloids (metal(loid)s) at the SWI, the analytes of interest in the sediment pore water have to be studied in parallel to different sediment parameters. Several methods for an active (e.g., suction, squeezing or centrifugation), or a passive (e.g., dialysis or DGE/DET) pore water sampling are available. These often require the installation of the sampling devices at the sampling site and/or intensive preparation procedures (e.g., slicing and centrifugation or re-elution from/digestion of the accumulation gels), probably impacting the environmental conditions at the area studied and/or the characteristics of the samples taken. The authors developed suction based, direct and low invasive sampling methodologies that enable to study element distributions in sediment pore water at the SWI at different spatial resolutions (sub-mm to cm) and depths (2 cm – 20 cm within the sediment). By combination of this micro or meso sampling system with a mircroprofiling system (Missy/Messy) and different sample preparation procedures, it was possible to address the fractionation (size dependent and micelle mediated) as well as the speciation of metal(loid)s in parallel to different sediment parameters (O_2, redox and pH) along sediment depth profiles. The profiling experiments were complemented by slurry reactor experiments enabling to simulate specific conditions by controlling the redox potential. The vision of the experimental approaches is to address a variety of parameters in parallel and, thereby, to help to deliver a more holistic understanding of natural and anthropogenic caused processes that govern the fate of substances at the SWI. Within the presentation, the results of different Missy and Messy experiments will be given. The different approaches will be discussed, especially with regard to their potentials and limitations and in comparison to other methods.

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Chromium speciation and release from mining waste in Barro Alto and Crominía, Goias state, Brazil
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Chromium is abundant and naturally present in ultramafic rocks and serpentine

soils. However, anthropogenic activities such as mining, leather tanning, chrome plating and metallurgical industries release an important amount of chromium into the environment. In spite of existing in chemical forms displaying oxidation number from 0 to VI, only hexavalent (Cr(VI)) and trivalent chromium (Cr(III)) are stable enough to occur in the environment. Despite Cr(III) is not toxic and more stable under natural conditions, Cr(VI) is known to be highly soluble in water, bioavailable and toxic. In plants the toxicity of Cr(VI) results in the suppression of seed germination, chlorosis of its leaves and oxidative stress (Choppala et al., 2013). In human and animals, the dermal contact is the most important exposure pathway, and as a result Cr(VI) easily crosses physiological barriers, enters into the blood stream, and destroys the red corpuscles (Jacobs and Testa, 2005). In order to determine the potential release of Chromium from mining activities and its impact on the environment, chemical extraction, chemical leaching under controlled physico-chemical conditions and isotopic exchange techniques associated with mineralogical studies has been applied in solid samples (soils, ores and sediments) collected in two areas located in Goiás State (Brazil). The first site is Barro Alto, Ni mining and metallurgic area, and the second is the Crominía, old chromite exploitation area. The chemical exchangeable Cr(III) and Cr(VI) were extracted with KCl 1M and KH_2PO_4 0,1 M respectively. Controlled leaching experiments were carried out with a $NaNO_3$ 10^{-3} M and NaN_3 0.02% leaching solution (S/L ratio of 20:1), controlling Eh and monitoring other parameters (pH, DO, Fe, Anions, etc). In addition, isotopic exchange was performed with ^{50}Cr (4.35% of abundance). The samples obtained from the chemical extraction and the isotopic exchange were all analyzed with HR-ICP-MS (ThermoScientific Element-II). Additionally, X-ray diffraction (XRD) analyses were performed on soils powder and shown that the mineralogy of the ores and soils was dominated by iron oxides. X-ray fluorescence (XRF) and acid digestion with Inductively coupled plasma atomic emission spectroscopy (ICP-AES) analysis were used to assess the total element concentrations of the solid samples. The results of the chemical extraction and ionic chromatography indicates that exchangeable Cr is mainly under hexavalent form.

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Do climate conditions affect metal bioaccumulation in earthworms?

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The present study aimed to assess the effects of changing climate conditions (temperature and soil moisture content) on the bioaccumulation of Zn and Cd in the earthworm *Eisenia andrei* in soils polluted by metal mining wastes. Bioassays were performed in different metal-polluted soils (mine tailing and watercourse) and under different combinations of temperature (20 and 25 °C) and soil moisture content (50 and 30% of the soil water holding capacity - WHC). Adult earthworms were individually exposed to the study soils for 21 days and analysed for internal Zn and Cd concentrations at different times. Before and after 21 days of exposure soils were analysed for pH and metal concentrations in porewater. The mine tailing soil did not show changes in metal availability (Zn-1, Cd-1). In the watercourse soil, porewater concentrations of Zn and Cd significantly decreased after 21 days exposure to the different climate conditions tested (Zn: from ~3761 to ~1613-2170 $\mu g\ L^{-1}$; Cd: from ~63 to 32-41 $\mu g\ L^{-1}$), especially at 20 °C and 50% of the soil WHC (standard conditions). In the latter soil, metal availability decrease was accompanied by a significant pH increase at 20 °C and 50% of the soil WHC. Both study soils showed a similar Zn bioaccumulation pattern in earthworms, without differences among climate conditions. Earthworms reached the highest internal Zn concentration after 1 day of exposure (~450-1500 $\mu g\ g^{-1}$ d.w.) then remaining constant until the end (typical pattern of essential elements). The Cd bioaccumulation pattern changed when changing the climate conditions. At 20 °C and 50% of the soil WHC the internal Cd concentration continuously increased from ~4 to ~13-19 $\mu g\ g^{-1}$ d.w. (typical pattern of non-essential elements). But when increasing temperature and/or decreasing soil moisture content the pattern changed towards a pattern typical of essential elements due to increased Cd elimination rates (mine tailing: from ~0.11 to 0.24-1.27 d^{-1}; watercourse: from ~0.07 to 0.11-0.35 d^{-1}) and faster achievement of a steady state. This study shows that soil incubation for 21 days under controlled conditions decreased Zn and Cd availability in metal-polluted soils, with higher decreases at 20 °C and 50% of the soil WHC. Changing climate conditions modified the bioaccumulation pattern of Cd in *E. andrei* exposed to metal-polluted soils, with faster achievement of the steady state due to higher elimination rates.

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Keeping memory of past mining activities: assessment of trace metal bioavailability and ecological risk using active biomonitoring.

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Mining activities are the first substantial source of trace metals (TM) contamination of the whole environment. However, past mining activities are often forgotten or minimized due to the disappearance of visual marks while mining wastes are known to be highly concentrated in TM. Bioavailability of TM and risk assessment linked to past mining and smelting activities are developed in a past mining district in the Vosges Mountains (France) to reconstruct their environmental impact in a forested valley. A bioindication campaign using the land snail *Cantareus aspersus* as bioindicator of TM bioavailability was conducted for six weeks along a gradient of soil TM concentration with eight stations comprising two archaeological mining stations, a communal garden, four forested stations and a control station. Three microcosms were placed in each station with fifteen snails per microcosm. Each week, two snails were randomly sampled for digestive gland TM analyses. Bioavailability of TM is evaluated by the uptake rate of TM. Bioavailability of TM is high in the eight stations compared to recent industrial sites, highlighting the importance of knowledge of localization of past mining and smelting sites. Moreover, the two archaeological mining sites present the highest bioavailability of TM to snails with, for example, 68.40 $\mu g_{Pb}.g_{dg}^{-1}$dry weight.d^{-1} and 0.027 $\mu g_{Cd}.g_{dg}^{-1}$dry weight.d^{-1}. A high bioavailability of TM is observed in the communal garden. These results conduct to assess the risk of this past contamination by using two indicators, i.e. SET and ERITME index which allow assessing the excess of transfer and the toxicological risk linked to TM. Excess of transfer and toxicological risks linked to archaeological mining sites are the highest ever measured with these index. Those calculated for the communal garden are similar to those on recent industrial sites. In conclusion, this study highlights that time elapse since the deposit of contaminated particles from mining and smelting activities is not sufficient enough to strongly catch TM in soils without transfer to trophic chain.

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Water quality assessment of a catchment affected by historical lead mining

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Historical metal ore extraction and processing have resulted in severe water quality issues worldwide. In England and Wales approximately 20% of water bodies fail the objectives of the Water Framework Directive (WFD). Environmental agencies from the United Kingdom and the European Union are constantly challenged in the implementation of this directive, therefore applied research and monitoring programmes are essential for the understanding of metal ion speciation, mobility, accumulation and bioavailability in river ecosystems. In northern England an exhaustive study has been carried out in an upland catchment polluted by heavy metals from historical mining. This research aims to identify the sources of pollution in the catchment and overall significance to water quality criteria for better management and potentially remediation of surface and ground water. A multi-methodological approach has been applied including water quality monitoring and conventional chemical analyses (Ion chromatography, ICPMS) for determining metal sources and concentrations. Correlation tests and principal component analysis have provided a consistent physicochemical characterization of the catchment, reflecting different sources of minerals that affect its water quality. Results suggest that the major geochemical signals are determined by the contribution of carbonate host rock (Ca^{2+}-Mg^{2+}:DIC:EC r=0.9, p< 0.001), other metals are possibly associated with weathering (Fe^{3+}- Al^{3+} -Pb^{2+}-Cu^{2+}:DOC r=0.2-0.7, p< 0.001) and erosion of mined ores such as sphalerite (ZnS) and galena (PbS)(Zn^{2+}-Cd^{2+}:$SO4^{2-}$ r=0.6-0.7, p< 0.001). Maximum concentrations of Zn^{2+}, Pb^{2+} Cd^{2+}, and Cu^{2+} (7438.4 mg/l, 439.9 mg/l, 47 mg/l, 19.8 mg/l) exceeded the Environmental Quality Standards by 99x, 61x, 313x and 2x, respectively. In addition, the identification of different flow regimes emphasizes their impact on metal discharge. Accordingly, mine channels are considered as important point sources, but a major problem is caused by extensive mine tailings during high flow scenarios becoming significant sources of diffuse metal pollution. Results suggest that a catchment-scale assessment combined with analytical approaches offer reliable information about factors controlling metal inputs (point and diffuse), providing monitoring programmes with a practical way to evaluate heavy metal pollution in headwater catchments.

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A field-based approach to linking biological responses of freshwater organisms to sediment contamination by metals

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The legacy of contaminated sediment is a serious impediment to rivers meeting the water quality targets set out by the EU Water Framework Directive. Whilst controlled ecotoxicity studies in the laboratory can provide an indication of the sensitivity of biota to sediments contaminated with metals, it can be difficult to relate this to the response of organisms in the field. Under field conditions, a variety of factors can influence the uptake of contaminants, such as metals, from the sediment by biota. These influences can be difficult to accommodate when deriving environmental limits that are relevant to field conditions. Here we have used a GIS approach based on river reaches to match biological data, from Water Framework Directive monitoring programmes of diatoms, invertebrates, fish and macrophytes, to a synoptic survey of riverbed sediment chemistry. A total of 2833 sites were identified with matching sediment and biological data. A threshold biological response to sediment metal concentrations was expected, where the metal would have no influence until a threshold concentration had been exceeded. By modelling the 95%ile, biological response thresholds to sediment concentrations of the metals silver, cadmium, chromium, copper, nickel, mercury, lead, tin and zinc, and the metalloids arsenic and antimony were determined. Based on field data describing the response of the relevant BQEs, the rank order of sensitivity to sediment contaminated with metals and metalloids (from most sensitive to least sensitive) was diatoms < invertebrates < fish < macrophytes. Despite the uncertainty involved in the data matching exercise used to produce the datasets, these findings, based on field data, suggest that for some metals the existing sediment quality guidelines may be too precautionary.

Toxicity Testing in Sediments - Bioassays As Link Between Chemistry and Complex Benthic Community Testing for Sediment Quality Assessment

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Can transcriptomic biomarkers worthwile enhance sediment-contact tests by molecular biomarkers?

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In sediment-contact tests effects caused by sediment-bound pollutants usually are assessed on a macroscopic scale. In this approach a sediment-contact test with rice is enhanced by molecular biomarkers for arsenite contamination. As macroscopic endpoint the inhibition of root elongation was assessed. A dose-response curve for the inhibition of root elongation was characterized and the EC_{50} was calculated as 13 $mg{\cdot}kg^{-1}{}_{dw}$. Molecular endpoints in form of transcript biomarkers were derived from microarray analysis. For this purpose, rice plants were exposed to two arsenite concentrations, namely 11 $mg{\cdot}kg^{-1}{}_{dw}$ and 15 $mg{\cdot}kg^{-1}{}_{dw}$ on spiked artificial sediments. Differentially expressed genes that are dose-dependently induced were selected as candidate biomarkers. From these genes a subset of five genes was analyzed by means of qPCR in rice roots that were exposed to a broader range of arsenite concentrations resulting in gene-specific dose-response-relationships. The five candidate biomarker genes were also characterized after exposure of rice to natural sediments with arsenic concentrations ranging from 13 to 23 $mg{\cdot}kg^{-1}{}_{dw.}$ On arsenite spiked artificial sediments, all genes showed a dose-dependent increase of expression up to a maximum at 9 or 13 $mg{\cdot}kg^{-1}{}_{dw}$ respectively. While on artificial sediments a distinct inhibition of root elongation was observed, on natural sediments only at the highest concentration of arsenic, an inhibiton of root elongation occurred. This finding from the natural sediments contradicted the expectations based on the results of the artificial sediment where an inhibition of the root elongation was observed at lower concentrations. This might be explained by the observation, that rice roots grown on silty natural sediments like, grow thinner, but longer compared to roots grown on sandy artificial sediments. However, one of the selected molecular biomarkers reacted clearly to the contaminated sediment. These findings might suggest that the macroscopic endpoint root elongation is less sensitive on silty natural sediments compared to molecular endpoints, if sandy references sediment is used. In sum, it was possible to identify candidate biomarker genes for arsenite stress by means of an omic-approach. On artificial, but especially on natural sediments, the biomarker react more sensitive than the inhibition of root elongation and thus indicated a stress response before adverse effects become manifest on a macroscopic level.

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Evaluation and selection of test methods for the assessment of contaminated sediments in the Baltic Sea - The CONTEST project

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There is a growing need to establish reliable, scientifically-based assessment methods to assess and classify the degree of contamination in sediments. This is necessary for decision-making concerning regulatory actions towards the reducing of pollution from different sources, removal and re-location of contaminated materials, and on-site remediation measures and the follow-up of these. The purpose of the CONTEST project ("Evaluation and selection of test methods for assessment of contaminated sediments in the Baltic Sea", 2014-15), funded by the Nordic Council of Ministers and the Finnish Ministry of the Environment, was to test, evaluate and select suitable biological methods for the quantitative and qualitative assessment of toxicity of anthropogenically contaminated sediments in the Baltic Sea marine environment. In the project, a battery of biotests currently in use in toxicity assessments were applied using a heavily contaminated harbour sediment as the test matrix. Chemical analysis of the sediment confirmed the presence of high concentrations of polycyclic aromatic hydrocarbons, organotins and trace metals. For the toxicity testing, a series of dilutions was prepared by adding reference sediment to the contaminated one, according to the toxicity results obtained from a preliminary toxicity test using amphipods. Various biological endpoints were examined in different test species, most of them ecologically relevant for the Baltic Sea. The following biotests/endpoints using whole sediments or sediment elutriates were applied: amphipod survival, larval development and biomarkers; gastropod survival, embryo development and imposex; copepod survival and larval development rate; water fleasurvival; sediment avoidance (amphipods); bacterial luminescence; micro- and macroalgae growth inhibition; SOS/umu genotoxicity. The majority of the tests applied showed concentration-dependent negative effects on the test organisms. Ranking of the biotests for recommendation purposes was based on the sum scores of a set of carefully formulated individual assessment criteria applied for each test/endpoint.

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Bioassays in framework of a triad assessment method for brackish sediments

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In sediment risk assessment, analysis of the pollutant concentrations is essential in determining the degree and nature of sediment contamination. However, chemical analyses provide no evidence of biological effects as, for example, mixture effects are not accounted for. Bioassays provide valuable information on the toxic effects of sediments, but are carried out under lab conditions and are limited to the testing of a few organisms. An evaluation of the benthic community structure can provide evidence of effects in situ. The sediment quality triad method incorporates measures of various chemical parameters, toxicological effects and benthic community structure in view of conducting an integrated assessment of sediment quality. In framework of developing a triad assessment method for the quality evaluation of brackish sediments, 28 sediment samples were taken along the Scheldt estuary (Sea Scheldt (Flanders) and Western Scheldt (the Netherlands)) and other brackish aquatic systems in Flanders. For these samples, chemical variables (e.g. metals and organic pollutants), toxicological effects and benthic community structure are being assessed. In this study, two sediment contact bioassays and a pore water bioassay are carried out to test their suitability for uptake in a quality triad method for brackish sediments as indicator of ecotoxicological effects. A 28-day sediment contact bioassay with the polychaete worm *Hediste diversicolor* is performed on a selection of the samples, with growth and mortality being evaluated. A 10-day sediment contact bioassay with the amphipod *Corophium volutator* and a 24h pore water test with the rotifer *Brachionus plicatilis*, evaluating mortality, are carried out on all the samples. For the sediments already evaluated mortalities vary between 0%-20% for *H. diversicolor*, between 1%-20% for *C. volutator* and between 10%-43% for *B. plicatilis*. Chemical concentration data are evaluated and compared with existing Sediment Quality Guidelines to assess the expected potential of sediment pollutant concentrations to influence sediment toxicity, and compared with the bioassay results. Through multivariate analysis the chemical, ecotoxicological and biological sediment measures are being analysed to assess the correspondence among the three components.

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(Dis)Agreement between different Lines of Evidence (LoEs) in the risk-based approach of dredged sediments

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The importance of sediments for the assessment of the quality status of aquatic environments has been thoroughly recognized and sediments monitoring is included in many programmes for the assessment of the quality status of marine environments worlwide. In addition, the assessment of the risk associated to contaminants present in the sediments plays a key role in the management of human activities in coastal areas, particularly dredging and relocation of sediments in ports. The results obtained from studies carried out for the management of dredging activities, in addition to those obtained from monitoring programmes that include sediments, represent a good opportunity to study relationships between different lines of evidence (LoEs) to assess the ecological risk of sediment-bound contaminants In this work we study the relationships between the sediment chemistry, the toxicity testing and the status of biological communities, in order to a) assess the degree of (dis)agreement among these three LoEs; b) identify the factors and conditions more likely distorting the expected cause-effect relationships; and c) suggest improvements in the risk-based approach for the management of dredging operations in the coastal areas of Spain. Data from several studies carried out by AZTI in the Basque coast (N. Spain) for different institutions were considered in this work. Overall, almost 300 sediment samples collected along the Basque coast with chemical information of contaminants (heavy metals and organic contaminants) and, at least, one biological LoE (toxicity testing and/or benthic communities) were considered. Most of the metal concentrations in the sediments were well above the ERL, and values above ERM were frequent, suggesting that adverse toxic effects were likely to occur. Statistically significant correlations have been found between variables of the three LoEs: e.g. %organic matter, PCBs, Cr, Ni and Zn are correlated with AMBI, Microtox® and anphipods toxicity. The quality status of the benthic communities was not clearly related to the others LoEs. This indicates the complex relationships between the chemical and the biological compartments.

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A weight of evidence approach for assessing remediation of contaminated sediments using food web tissue contamination, biotic condition and DNA damage.

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The Ottawa River drains northwestern Ohio, flowing into Lake Erie's western basin at the city of Toledo. The Ottawa River is a component of the Maumee River Area of Concern (AOC) as defined by the International Joint Commission's Great Lakes Water Quality Agreement. A sediment remediation project took place in the lower 14.2 km of the river where urban and industrial activities impacted the river as a beneficial resource. Sediment was removed based on a surface weighted average concentration model where PCB and PAH levels exceeded targeted levels. This presentation will focus on three biological tools used to assess remedy effectiveness: change in fish, invertebrate and Tetragnathid spider tissue concentrations of PCBs and PAHs, DNA damage in Brown Bullhead and macroinvertebrate biotic condition as measured by Ohio EPA Lacustrine Index of Community Integrity (LICI). From 2009-2013 and again in 2015, pre- and post-remedy sampling of fishes representative of different trophic levels was conducted via electroshocking and fyke net sampling. The study area was divided into three river reaches with distinct hydrogeomorphology. Fish collected from each reach included: Largemouth Bass, Brown Bullhead, White Sucker, Pumpkinseed, Gizzard Shad, Bluntnose Minnow and Emerald Shiner. Blood samples were collected from 10 Brown Bullheads from each reach to assess DNA damage using Comet Assay methods. Multi-plate samplers were deployed for collection of macroinvertebrates for tissue analyses and biotic condition assessment. DNA damage in Brown Bullhead increased during dredging then declined in subsequent years. Largemouth Bass, White Sucker, Brown Bullhead, Pumpkinseed, Bluntnose Minnow, macroinvertebrate & spider tissue concentrations showed no change 3-y post dredging compared to pre-dredging. Gizzard Shad and Emerald Shiner showed lower tissue concentrations 3-y post dredging compared to pre-dredging across the entire project area. No difference in the LICI was found based on samples collected before and after dredging. Based on modeling performed during the design phase, it was anticipated that the long-term clean up goals would be met approximately 10 years after the completion of dredging activities, by 2020.

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A comparative approach using ecotoxicological methods from single-species bioassays to model ecosystems

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Effects on natural nematode assemblages to chemical stress (Zn) were assessed in this study in complex sediment microcosms, including indirect food-web effects, and in acute community toxicity tests, considering direct toxicity only. Responses of the various freshwater nematode species in both approaches were compared to effects of Zn on the well-established model organism *C. elegans* in standardized toxicity tests (ISO 10872). The acute tests were performed with a concentration of 20 mg Zn/L, resulting in a sensitivity ranking of *in situ* nematode species. The findings only partly reflected classifications of nematode species according to the NemaSPEAR[%]-index but underlined the function of *C. elegans* representing the sensitivity of freshwater nematodes. The sediment in the microcosms was spiked with nominal Zn concentrations of 10 and 100 mg Zn/kg Sediment (dw). Over the course of the study, Zn had strong dose-dependent effect on nematode abundance, species richness, and species composition, as well as on the NemaSPEAR[%]-index,with significantly lower values in HD-microcosms. Additionally, standardized *C. elegans*-toxicity tests with whole sediment samples and filtered pore water were conducted to estimate bioavailability and direct toxicity, indicating that concentrations causing sublethal effects in *C. elegans* have already severe effects on natural nematode communities. Even at relatively low pore water concentrations of 1 mg Zn/L, corresponding a 20% inhibition of reproduction of *C. elegans* only, striking effects on the natural nematode assemblages could be observed. By this study, a better interpretation of toxicity bioassays in terms of sediment quality assessment was aimed. Whereas the function of *C. elegans* as representative of nematodes could be confirmed when comparing its sensitivity to common freshwater species, results of the more complex microcosm approaches underline the importance of considering also indirect (food-web) effects. Combining sophisticated experimental tools with field observations allows for more accurate decision making in environmental risk assessment. Data on chemical concentrations, single-species and community toxicity, and in-situ assemblages can be integrated as single lines of evidence in a weight-of-evidence approach. As suitable ecotoxicological tools and ecological indices (NemaSPEAR[%]-index) for nematodes are already available, this organismal group should be used more often in sediment quality assessments.

Advances in exposure modelling: bridging the gap between research and application (II)

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Exposure to Chemicals in Consumer Products: The Role of the Near-Field Environment

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Humans can be exposed to chemicals in consumer products during product use and environmental releases with inhalation, ingestion, and dermal uptake as typical exposure routes. Nevertheless, chemical exposure modeling has traditionally focused on the far-field with near-field indoor models only recently gaining attention. Further, models that are mostly emissions-based, may not necessarily be applicable to all types of chemical release from consumer products. To address this gap, we (1) define a framework to simultaneously account for exposure to chemicals in the near- and far-field, (2) determine chemical product concentrations for various functional use categories, (3) introduce a quantitative metric linking exposure to chemical mass in products, the Product Intake Fraction (PiF), and (4) demonstrate our framework for various consumer product categories. This framework lends itself to high-throughput calculations for characterizing exposure to the vast consumer product chemical space. The chemical mass in products is used as a starting point for quantifying human exposure obtained by multiplying the chemical concentration (e.g. % w/w) in the product with the amount of product used per defined application. Chemical concentrations in products can be obtained from empirical studies, formulations and associations described in databases, or when unavailable, estimated based on chemical-product functions or regulatory frame formulations. Exposure is quantified by estimating the PiF, the fraction of the chemical in a product that is taken in by humans via each exposure pathway, considering specific compartments of entry into the near-field environment (releases of chemicals encapsulated in articles, indoor air spray, etc.). To estimate PiFs, we combined far-field environmental compartments with near-field compartments and exposure pathways in a multimedia matrix of transfer fractions, with columns and rows for each compartment and exposure pathway. The multiple transfers and PiFs (e.g. from chemicals encapsulated in articles to inhalation of indoor air and dermal uptake via skin contact) were obtained by inverting the transfer fractions matrix, yielding cumulative multimedia transfer fractions. PiFs for various chemicals in products were found to be on the order of $1x10^{-7}$ for semi-volatile organic compounds (SVOCs) in thick flooring, $5x10^{-3}$ for VOCs in indoor air spray, and up to 95% or even higher for ingredients in leave-on cosmetic products.

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Junge relationships in modelled and measured concentrations of chemical pollutants in the Danube River

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Persistence is an undesirable property of chemicals that is difficult to measure in the real environment. Junge relationships describe the correlation between the relative standard deviation of concentrations of chemicals and their residence times. Once calibrated for a set of compounds they can potentially be used to estimate the persistence of pollutants. The parameter *b* in the Junge equation is a measure of the strength of the relationship between variability in concentrations and residence time.[1] In this study we explored Junge relationships relating the concentrations of chemical pollutants to their degradation half-lives in the Danube River. Concentrations of four hypothetical chemicals with biodegradation half-lives of 7, 15, 30 and 90 days were obtained using the STREAM-EU model [2]. Junge relationships were calculated as 1) "Temporal" relationships from variability during the year at each of the stations, and 2) "Spatial" relationships from variability between the stations on each day of the year. Next, a Junge relationship was calculated from the data of the 2nd Joint Danube Survey (JSD)[3] for caffeine and six pharmaceuticals: benzafibrate, carbamazepine, diclofenac, gemfibrozil, ibuprofen and sulfamethoxazole. Half-lives of the compounds were gathered from literature. We found Junge relationships in the Danube in both modelled and measured concentrations. Results from STREAM-EU show clear Junge relationships for the 4 theoretical chemicals. The parameter *b* ranges between 0.1 and 0.8 for the temporal analysis and from 0.1 to 0.4 for the spatial analysis. The higher *b* values in the temporal analysis are found downstream (i.e. Romania), were population and emissions are relatively low. For the spatial analysis, the higher *b* are found in the month of November. The analysis of pharmaceuticals and caffeine data from the JSD, also shows a Junge relationship with *b* of 0.25, well within the values obtained in the modelled spatial Junge relationships. Modelling results suggest that Junge relationships are more likely to be found during November and downstream in the Danube, but this has not been confirmed with monitoring data. Measurements within the optimal spatial and temporal conditions defined by the modeling analysis could potentially be used to estimate the persistence of other chemical pollutants. [1]MacLeod, et.al. 2013. Chemosphere 93:830-834[2]Lindim, et.al. 2016. Chemosphere 144:803-810[3]ICPDR. 2007. http://www.icpdr.org/wq-db/home

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Application of a spatial model to contextualise monitoring data for risk assessment of down-the-drain chemicals over large scales

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Regulatory screening level exposure assessments (e.g. EUSES) are based on simple conceptual scenarios at various scales. However, exposure, and therefore risks associated with chemicals, are characterised by high spatial variability. The Scenario assembly tool (ScenAT) is a global screening level exposure model to enable spatially explicit predictions of environmental concentrations of home and personal care chemicals. It uses a simple equation to predict local scale freshwater concentrations (PECs) of chemicals discharged via wastewater, as described in the EU TGD. Calculations are performed for 28,080 spatial units across 88 countries worldwide using GIS data layers for the underlying socio-economic (population, GDP) and environmental parameters (per capita water use, STP connectivity, dilution factor). Using a probabilistic approach, we incorporate sources of uncertainty in input data (tonnage estimation, removal in sewage treatment plants and seasonal variability in dilution factors) into spatially explicit model simulations for two test chemicals: the antimicrobial triclosan (TCS) and the anionic surfactant linear alkylbenzene sulphonate (LAS). We then compare model estimates of wastewater and environmental concentrations of TCS and LAS to monitored data available for the UK. This comparison showed that screening-level modeled probabilistic PECs were higher than mean measured data for TCS and LAS by a factor 3.4 and 1.4, respectively. Considering the uncertainty associated with both model and monitoring data, results suggest that the use of a probabilistic approach using the ScenAT model for screening assessment over large scales is a valid approach, produces output that is consistent with monitoring data, and thereby represents a robust screening level exposure assessment tool. The combination of modelled and monitoring data enables the contextualisation of available monitoring data, focussed on few specific catchments, over larger scales. Such information can be used to support the refinement of exposure assessments as well as for the identification of areas of high concentration for use in higher tier assessments.

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Exposure hotpots and source apportionment analysis of home and personal care products chemicals in Asia

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The increasing use of household and personal care (e.g. HPC) products in developing countries requires new tools to assess the potential ecological impacts of HPCs, accounting for local hydrological and use characteristics. This paper therefore aims a) to develop a tiered set of models to describe the hydrological and multimedia fate of releases associated with the use of HPCs in Asia b) to combine the fate model with georeferenced emission scenarios to predict chemical concentrations and to identify of (sub)catchments hotspots, and c) to perform an apportionment analysis to understand dominant near and distanced sources. Geo-referenced emissions scenarios are first determined for the entire region using the ScenAT model and focusing on six different test substances. These emission scenarios are then used as input to the regionalized USEtox (tier 1) and to the Pangea fate and exposure model (tier 2) that has the ability to create scenario-specific multi-scale grids. A more detailed analysis is carried out on the Yangtze and Huai River catchments in China, comparing estimated exposure hotspots with water quality grades. LAS estimated concentrations in freshwater are the highest among the considered HPC chemicals but few LAS monitoring data are available for Asia. The higher resolution of tier 2 leads to higher spatial variation in water concentrations. Of special interest is the contrast between short and long lived chemicals in surface water. For a short-lived substances such as LAS (half-life of 0.29 d), dilution leads to decreasing concentrations in the Yangtze River as we move downstream, whereas concentrations are relatively constant for a less degradable substance. The highest concentrations are observed for the Ying River, a tributary of the Huai River, classified with the poorest grade (V+) for macro indicators of water quality in China. For LAS only a few adjacent cells substantially contribute to the concentration in the terminal cell, 63% of the concentration being due to direct emissions in this terminal cell. In contrast, for triclosan, only 1% of the concentration in the terminal cell is due to direct emissions, 5% from neigbouring Shangai, and the remaining 94% coming from more than 220 cells spread over 2000 km upstream in the catchment. The combination of ScenAT with the multi-scale multimedia model Pangea provides a parsimonious way to identify hotspots and to study them with refined resolution.

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Countrywide risk assessment concerning the exposure of watercourses to spray drift in fruit growing in the Netherlands

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For aquatic organisms, the risk of exposure to pesticides depends highly on deposits of spray drift onto surface waters. Downwind off-target deposits of spray drift have been investigated for many years. In fruit crops, pesticide sprays are applied in an upward or sideways direction. Consequently, downwind deposits are significantly higher than those for field crops, where sprays are applied in downward direction. For field crops, various spray drift models have been described in literature. For fruit crops no models are available to assess the exposure of surface waters to spray drift. Recently, a generic spray drift model for pesticide applications in pome fruit crops has been developed based on a large set of experimental data. The model is implemented in an exposure assessment model to estimate pesticide concentrations in all edge-of-field watercourses next to fruit orchards in the Netherlands. The exposure assessment model accounts for a large number of spatially and temporally varying conditions. These include the topography of orchards and their orientation, the spatial distributions of edge-of-field watercourses and their geometry, spatio-temporal frequency distributions of weather conditions (typically, wind speed, wind direction and ambient temperature). Another important factor is the growth stage of the trees at the days of pesticide application. Drift reducing application techniques and multiple spray applications during the growing season are accounted for. All of these features result in an exposure assessment model with a high level of realism. In an extensive simulation study the predicted environmental concentrations (PECs) in the watercourses were computed for all possible spatial configurations. A spatio-temporal statistical analysis on these simulations resulted in a quantitative risk assessment for a representative set of spray application schemes. Currently, the present model is combined with a model describing the fate of pesticides in edge-of-field watercourses. In this way, a realistic simulation study on the fate of pesticides in surface waters can be performed to quantify exposure risk levels for aquatic organisms. This serves higher-tier assessment studies for the authorization of plant protection products.

Risk Assessment of Biocides - latest developments

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Introductory talk: Overview on the environmental risk assessment for biocides and latest developments of guidance and guidance-related documents

H. Schimmelpfennig, ECHA-European Chemicals Agency / D Biocides

SETAC session: Risk Assessment of Biocides- latest developments, under main track: Risk assessment, mitigation and monitoring **Short abstract Introductory talk: Overview on the environmental risk assessment for biocides and latest developments of guidance and guidance-related documents** Heike Schimmelpfennig, Eugenia Nogueiro, Anna Wik, Simon Gutierrez Alonso ECHA, Annankatu 18, FI-00121 Helsinki, FINLAND **Key words**: environmental risk assessment, biocides, guidance development The aim of the introductory talk is to set the scene on how environmental risk assessment is defined for biocides, on how it is currently performed, where to find relevant guidance documents as well as to provide an overview on current ongoing guidance developments. According to Annex VI of the BPR, any risks arising from the use of a biocidal product needs to be identified, therefore a risk assessment is carried out for the different product types (PTs) to determine the acceptability of the claimed use or otherwise of any risks that are identified. Detailed information on how to perform an environmental risk assessment for biocides is provided on the ECHA webpage. The development of guidance and or guidance-related documents like the ESDs is still ongoing. The following guidance documents have been finalised since entry into operation of the BPR (09/2013): Mesocosm guidance (available as Appendix in Vol. IV Part B) Cut off criteria for groundwater assessment of biocides Volume IV Part B: Guidance on Environmental Risk Assessment – Active Substance Scenario for the biocidal use and emissions from oil platforms PT11/ PT12 for PEC calculation Direct emissions to surface waters in PT 6, 7, 8, 9 and 10 Use scenarios for PT 9 roof membranes Supplement to the ESD for PT 13 Emission Scenario Document for PT 19 PT 21: Fish net scenario in aquaculture The preparation of the following guidance documents is currently coordinated by ECHA, member states are actively involved in the preparations: PT 8: Acceptability of the current methods to assess the exposure/risk of wood preservatives Proposals for standard scenarios and parameter setting of the FOCUS groundwater scenarios when used in biocide exposure assessments Leaching to groundwater from paint, coatings and plaster Harmonisation of leaching tests for PT 7, 9 and 10 - test methods.

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Mixture toxicity assessment of biocides: Experimental verification of the relevance of additives in wood preservative products
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The Biocidal Product Regulation requests to consider combined effects of active substances and of active substances and relevant additives in the environmental risk assessment. The present study aimed to inform guidance development regarding the identification of additives as being relevant for such a mixture risk assessment. Furthermore it aimed to verify if theoretical mixture assessments can substitute for ecotoxicological testing of biocidal products or their residues released into the environment. The hypothesis of the study is that the consideration of the active substances and additives of environmental concern is sufficient to reliably predict the aquatic toxicity of biocidal products using the concept of Concentration Addition (CA). \nSeven wood preservative products were chosen for the experimental verification of the hypothesis using green algae (growth rate after 72 h), *Daphnia magna* (immobilisation after 48 h) and fish embryos (mortality after 48 h and/or 96 h). To determine how relevant various types of additives are for a protective mixture risk assessment, the Model Deviation Ratio (MDR) was calculated for different subsets of product components.\nIn general, the CA prediction was (over)protective for the so far tested products. If this can be confirmed as a general finding, the theoretical risk assessment of products by the CA concept appears sufficiently protective to be used as a substitute for product testing, taking only additives into account that are identified as environmentally relevant. The presentation aims to give an overview of the results of all seven tested products and will also discuss whether such findings can be generalized for the risk assessment of biocidal products in view of the limited variability of wood preservative products available for testing in the present study.

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Biocidal use of anticoagulant rodenticides results in the secondary exposure of non-target animals
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Residues of anticoagulant rodenticides (AR) in non-target animals were studied in Finland. ARs are transferred to non-target animals when they prey on poisoned rodents. In particular, second generation ARs (SGARs) have been found in non-target animals in many countries. This study focused on species which feed either on rodents or their carcasses and in which ARs have been found in other countries. The animals were either found dead or were shot or trapped for other purposes. Bromadiolone, difenacoum, brodifacoum, flocoumafen and coumatetralyl were analysed in 136 liver samples by an HPLC-triple quadrupole mass spectrometric method. One or more ARs were detected in 87% of samples. ARs were commonly found in eagle owls, tawny owls, raccoon dogs, foxes and mustelids. The most commonly found AR was bromadiolone which was also found in the highest concentrations. Bromadiolone was the most frequently used AR in Finland in 2014. The second most common AR present in the livers was coumatetralyl followed by difenacoum, brodifacoum and flocoumafen. Overall, the prevalence of ARs in non-target species correlated well with the sales of these substances. A high variation of concentrations was found within and between animals. In general, coumatetralyl was found in lower concentrations compared to SGARs. Our results show that the biocidal use causes frequent exposure of non-target animals, because ARs are authorized only as biocides in Finland and the use for the crop protection can be considered negligible. There are several restrictions on the use of ARs in Finland. The most restricted use is allowed to the general public while the professional pest control operators (PCO) have the widest selection of substances as well as a possibility to use ARs outdoors. Even the most restricted substances, i.e. those allowed only to indoor use by PCOs were found, but in lower frequency compared to more commonly used substances. Coumatetralyl was found surprisingly often despite of its anticipated low use and fast elimination half-life. On the other hand, concentrations of coumatetralyl in the baits were several times higher compared to other ARs. Majority of concentrations were assumed to be sublethal, but in 15% of non-target species concentrations were so high that ARs could have contributed to the death of these individuals.

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The fate of biocides in stormwater pond sediments
E.A. Rudelle, Aalborg University / Department of Civil Engineering; U.E. Bollmann, K. Bester, Aarhus University / Department of Environmental Science; A.H. Nielsen, Aalborg Unversity / Department of Civil Engineering; J. Vollertsen, Aalborg University / Department of Civil Engineering
Biocides are added to paints, wood preservatives, plasters, and other building materials to protect them against fouling. Upon application, biocides also ensure long shelf life of the product. However, biocides are slowly released, washed off during rain, and enter the stormwater flow, resulting in concentrations that routinely exceed quality standards set by the EC. Stormwater contains a range of other pollutants which are perceived as problematic. To mitigate their impacts, retention ponds are routinely used to treat the runoff prior to discharge into the environment. The objective of this study is to assess how selected biocides are sorbed and degraded in the organic-rich sediments of stormwater retention ponds. No study has hitherto reported the fate of biocides in retention ponds, and only few studies exists on pesticides in sediment from comparable systems. The sorption capacity, sorption kinetics, and degradation of Cybutryn, Terbutryn, Diuron and Carbendazim were investigated in microcosms simulating retention ponds. Stormwater sediments were deposited at the bottom of microcosms, and biocides were added. The removal of biocides from the liquid phase was monitored. After 3 weeks of incubation the sediments were extracted and analyzed for biocides. Parallel hereto sorption kinetics and stoichiometry was determined on the same sediments kept in suspension, the latter based on the relevant OECD standard. The total degradation of biocide was 10-30%, depending on biocide and sediment type. The sorption determined by slurry-tests and the microcosm tests did however not agree. The amount of biocide remaining in the liquid of the microcosms after 3 weeks were up to 10 times higher than what was expected based on the sorption capacities determined by the slurry tests. A fully dynamic diffusion-sorption-degradation box-model was applied to analyze this discrepancy. The simulations represented the measurements well and allowed dynamic distinguishing between degradadation and sorption. The study showed that all biocides underwent sorption and degradation in stormwater sediments to a degree where these processes will affect the final amounts discharged from retention ponds to receiving waters. It was furthermore observed that sorption kinetics of biocides in deposited sediments differed significantly from those of sediment slurries and that kinetics based on slurry experiments significantly over-estimated the sorption to retention ponds sediments.

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Implementation of an Environmental Monitoring of Biocides to Follow Consequences of their EU Risk Assessment Outcome
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The implementation of the Biocidal Products Regulation (No. 528/2012; BPR) causes changes in the application of biocidal active substances and their entry into the environment. For biocides included in the list of approved substances concentrations may increase while decreasing environmental levels are expected for substances with non-approval decisions or implemented risk mitigation measures. Such consequences may be proven by an environmental monitoring. However, in most current monitoring programmes biocides are not appropriately covered yet. Traditionally, e.g. in surface waters mainly plant protection products (partly also approved as biocides) and legacy chemicals are monitored. In this context, the German Environment Agency (UBA) initiated a project which aims at developing a comprehensive monitoring concept for biocides which also includes selected monitoring studies. The developed prioritisation approach provides lists for a biocides monitoring in all relevant compartments (e.g. monitoring in surface waters: water phase, aquatic biota, suspended particulate matter/sediments). For the implementation of the proposed biocide monitoring approach mainly cooperation with existing programmes is recommended. At least some biocidal compounds are already covered (e.g., for surface water monitoring according to Water Framework Directive obligations). All proposed monitoring activities should be organised in a stepwise approach (e.g. screening study, survey in different regions, and inclusion in a routine monitoring programme). In a further part of the project archived biotic and abiotic samples from the German Environment Specimen Bank (ESB) were used for exemplarily retrospective monitoring studies to identify possible spatial and temporal patterns of selected biocides. As case studies monitoring data from recent investigations covering freshwater samples will be presented: e.g., cybutryne and tebuconazole in aquatic suspended particulate matter and rodenticides in fish liver tissue. Time series reveal sporadically occurrence of rodenticides such as brodifacoum in fish and decreasing levels of cybutryne (previously used as preservative in construction materials). Examples support the assumption that changes of the use of biocides after approval/non-approval decisions or as consequence of risk mitigation measures can be followed by an environmental monitoring. Appropriate monitoring data would also allow to check the appropriateness of exposure

estimations (verification of model outputs).

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Biocidal active substances in households - reasons for the need to promote a sustainable use of biocides
S. Wieck, O. Olsson, Leuphana University of Lüneburg / Institute for Sustainable and Environmental Chemistry; K. Kuemmerer, Leuphana University Lüneburg / Institute of Sustainable and Environmental Chemistry
Households are a possible application site for a wide variety of biocidal active substances that fall under the Biocidal Products Regulation (EU) 528/2012 (BPR). However, the usage pattern of these substances there is widely unknown. Furthermore, the very same substances are used in products that are regulated by other regulations, too. As exposures resulting from different regulatory areas are currently not aggregated, the risks of these active substances will be underestimated. The objectives of the work presented here are therefore (i) to identify the biocidal active substances that can be found in households and the respective product categories they are used in and (ii) to describe the cases where biocidal active substances might enter the sewage system without being covered by the BPR and thus are not evaluated under its risk assessment scheme. Face-to-face interviews were conducted in 133 households in predominantly urban, intermediate and predominantly rural study sites in Germany. Members of private households were interviewed using a standardised questionnaire. Additionally, the products that were used in the households were registered with the help of a barcode scanner. Biocidal active substances were present in all households, eventhough not all posessed biocidal products, as the majority of uses of biocidal active substances was in washing and cleaning agents and personal care products, but not in biocidal products. Around 60 % of the registered applications of biocidal active substances do not fall under the risk assessment of the BPR. These can be active substances present in washing and cleaning agents, which are not assessed or approved for the use as in-can-preservatives. Furthermore, all biocidal active substances present in personal care products are not covered by the risk assessment of the BPR. The results show that gaps exist in the risk assessment of biocidal active substances. The attempt to solve the problem would require an extensive increase of complexity of risk assessments and their aggregation throughout all legislation. From our point of view, a better approach to reduce possible risks by these substances in general would be to limit their use to in fact essential usages. A sustainable use of biocides should thus be promoted to account for existing gaps in risk assessment.

Life Cycle Data and Modeling Developments - From Data Collection to Usage (II)

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How to complete LCA studies data collection in order to improve its quality?
E. Lees-perasso, CODDE; A. Roy, Bureau Veritas CODDE; J. Orgelet, Bureau Veritas CODDE / EcoDesign; J. Garcia, P. OSSET, SCORE LCA
LCA is a more and more common and widespread tool used to calculate the environmental impacts of products and services. This importance leads to an increasing need for reliability and transparency of data and results. In parallel, the multiplication of LCA studies as well as the growing complexity of reference documents and analysed systems leads to an increasing need of temporal and human resources. Most of these are allocated to the collection and research of data to assess the systems considered. The time needed to perform this collection and the assessment is even greater as some data may be missing, hard to find or even false. Therefore, it is necessary to define and use methods in order to fill those gaps. Those choices are essential and must preserve the relevance and transparency of studies while limiting the need for resources. This presentation aims at introducing the study performed on behalf of the ScoreLCA association concerning how to answer this need for reliable methods of missing data gap completion. This study aims at answering this need through three steps: Describing existing reference documents requirements in terms of missing data Identifying, describing and applying actual or ongoing methods aiming at solving the data missing gap Determining recommendations allowing the selection and application of chosen methods, depending on the studied system typology and the LCA study goals Concerning the requirements, the study enlightened the different logical schemes about their implementation in the different documents, depending on the activity sector, the age of documents, and the involved actors and documents objectives. This study shows that the actual developments go towards a greater harmonization and complexity of requirements. Concerning the methods, the study concluded that the existing ones cover quite well the need for identification, analysis and substitution of missing data. Though, those methods have different application scope and advantages and drawbacks, due to inherent or external reasons. The LCA practitioners' difficulties reside in the selection and application of those methods. To go further, the study concluded on the importance to gather and analyse feedbacks from the different stakeholders (practitioners, verifiers, study users), as well as the methods and requirements answer thestudies objectives. This could provide a clear synthesis to be used as a basis for the development or update of future reference documents.

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How to prioritize data collection based on uncertainty analysis: Application to the regionalization effort
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Life cycle assessment (LCA) practitioners spent a lot of their time on data collection. A smart data collection insures a good study quality with low uncertainty. One of the main challenge for them is to focus effort on data that can reduce the overall uncertainty. Regionalization is one of the ways to reduce overall uncertainty. Life Cycle Inventory (LCI) regionalization deals with increasing the geographic representativeness modelled in LCI. Life Cycle Impact Assessment (LCIA) regionalization deals with calculating regionalized characterization factors (CF) that account for the spatial variability of the receiving environment for some environmental impacts. To use regionalized CF, elementary flows (EF) should be spatialized (LCI spatialization). Integrating regionalization requires additional effort on data collection and needs to be prioritized. This work proposes an operational methodology for LCA practitioners to prioritize data collection for regionalization purpose based on uncertainty analysis in order to select impacts, processes and EF that need further data collection. Its relevance and applicability is illustrated by a case study. The proposed methodology is stepwise, iterative and takes into account decision maker requirements to set the target for uncertainty reduction. It allows among other to prioritize the effort between LCI regionalization (if LCI uncertainty predominant) or LCI spatialization (if LCIA uncertainty predominant). Global sensitivity analysis tools are used to identifying uncertain sources that are the biggest contributors to the result uncertainty and that need further data collection. They have been selected based on a trade-off between accuracy and operationalization. For uncertainty contribution analysis (UCA), tools such as Contribution To Variance based Monte Carlo results and coefficient of variation (CV) are used. The proposed methodology has been tested on an ecoinvent v3 process using the IMPACT World+ LCIA method to investigated which aspects of this process should be regionalized. For water use impacts on human health, the relative importance of LCI and LCIA uncertainties are compared based on CV values. Main results show that the effort for data collection should be focus on information to spatialize water EF in order to use less agregated CF with a lower spatial variability. This methodology is described for regionalization purpose but could be further adapted for global data collection prioritization.

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Influence of data choices in Life Cycle Assessment of waste management systems
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Environmental models, e.g. life cycle assessment (LCA), are widely used to support decision-making. An important factor for the reliability and usefulness of the models is to which extent they represent reality, i.e. the system of interest, which is influenced by the representativeness of the input data and the model itself. There are specific challenges for LCA of waste management systems related to the collection of representative data. Site-specific conditions affect the quality of the waste and the waste composition due to local waste separation and collection schemes, why there is a high need for site-specific data when modelling waste management systems. The objective of this paper is to evaluate the influence of input data choices in LCAs of waste management systems. The hypothesis is that data choices affect the LCA such that end result can vary with orders of magnitude. This is evaluated by analysing and comparing the spread of results calculated on basis of the modelling of a case study, where data combinations are implemented in the model. The case study is landfilling of Danish residual household waste with the functional unit being landfilling of 1 tonne of waste. A mechanistic landfill model was applied, where sub-processes are represented by modules and emissions are linked to the fractional and chemical waste composition. In the model, data choices were varied based on known ranges to represent different options for landfill gas and leachate generation and different types of technical collection and treatment solutions. A total of 864 data combinations were obtained, hence resulting in 864 results per impact category. From the preliminary results we saw a factor 2 spread between the minimum and maximum values of potential impacts of climate change, ozone depletion, ionizing radiation, fossil resource depletion and particulate matter. This highlights that data choices, even individual technology choices, influence the LCA results. By tracking the results back to its data combinations we found that the largest potential impact of climate change corresponded to a combination with high landfill gas generation and limited gas collection and utilization. Based on these preliminary results, it can be concluded that the choice and availability of representative data influence the LCA results, and thus that proper identification of the correct modelling choices is crucial within specific technologies.

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Comparing models in estimating additive emissions from plastic materials in

use

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The amount of additives in the total societal stock was previously estimated based on trade stastistics combined with product lifetime estimates and as detailed information "as possible" about the additive type, down to substances and their concentration in materials. Emissions for this amount was then estimated by two different emission models. The work presented here is a cross-comparison of these two models for calculating emissions of additives in polymer materials during the use phase of the materials. The purpose is to investigate if, at all, a rough model can be considered good enough. The actual product type used for the comparison is wall paint. An important reason for this selection was the fact that composition information for total volumes of wall paint was available through the chemical product registry at the Swedish Chemicals Agency. The substances included are functional additives in paint, i.e. preservatives, biocides, etc. Actually the cross-comparison could have been done also on a theoretical composition of some other product, but the overarching purpose of the work was to estimate the actual emissions of the additives at stake. The two models provide very varying results. When looking at the total emission estimate for the whole stock of plastics in the economy, it becomes clear that the rough model overestimates the emissions of additives when added together across all plastics and all additives. The advanced model, on the other hand, has been shown to provide a reasonably accurate estimate at least for specific substances (DINP from vinyl flooring) also when validated against concentrations in the indoor and outdoor environment. In this particular case the rough model overestimates emissions by a factor of 250. It is however highly uncertain to apply a similar factor for other materials and additives, as the emission ratio varies across several orders of magnitude. The rough model must therefore be considered "not good enough", while the advanced model needs further validation for more material/additive combinations.

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A visual solution to optimize the understanding and exploitation of metadata from Open Data sources in LCA

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Metadata is a fundamental piece for the discovery and management of input datasets and methods in Life Cycle Assessment-LCA. Collaborative initiatives among LCA practitioners, database developers, data suppliers and other stakeholders worldwide have brought to several forms of metadata developments to improve the transparency of unit process datasets and impact assessment methods, providing the necessary knowledge on the applicability and relevance of data, helping reduce uncertainties and ensuring the reproducibility of LCA studies. The general impression is that the LCA practice is getting more and more oriented towards Open Access thinking, in particular for the use of life cycle commodity databases. Building on such free knowledge, however, can imply a lack of standardization, and pose additional problems to the consistency and quality of shared data. Hence, efforts to improve and harmonize metadata could reduce such issues. Information to build databases or update characterization models may be retrieved from open access portals, which can be valuable tools. The value of such information can even increase by using and combining datasets coming from different data sources. However, metadata from such sources, when defined, are not always easy to understand and exploit. This intensifies the complexity of searching and filtering datasets, which is one of the major challenges for database developers. Moreover, gathering datasets from different sources complicates the data collection because of different metadata policies, types and languages. While open data portals are more and more tailored to store large amounts of metadata, they are less effective in making data available. Such portals are not usually prepared to allow users downloading datasets, and not all of them use open formats (e.g. CSV and XML), limiting the dataset reuse. Thus, beyond the necessity to increase their readability, metadata need to be better exploited for more consensual, replicable and transparent dissemination and use of life cycle data. To this end, a novel visual technique is proposed here to organize in effective and friendly ways all metadata related to a group of life cycle datasets. This choice is made because data visualisation techniques allow rapid assimilation and recognition of large amount of information. For validation, a case study is presented with focus on an integrated modelling framework for the characterization of life cycle impacts on ecosystem services.

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UNEP technical support on datasets review: a summary of learnings

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Introduction The UNEP/SETAC Life Cycle Initiative has developed the Global Guidance Principles for LCA databases [1] that describes guidelines for LCA databases to be interoperable and of adequate quality. To reflect thoses Principles, a review of 40 datasets has been conducted with UNEP support by independent LCA experts. Review process, timeline and criteria The whole effort had to be delivered in a set and limited timeline of six months and covered 40 datasets from emerging economies, 20 from Malaysia, 10 from Brazil and 10 from Thailand. The intent of the review was not to validate or not the sufficient quality of the datasets for publication, but rather to encourage improvements, either prior to publication or as part of a continuous improvement cycle. The developed review process consisted especially in i) d*eveloping a set of review criteria* for this specific effort, ii) establishing a *non-disclosure agreement (NDA)* between the data provider and reviewer and iii) providing a review report for each dataset that *assesses the presence, magnitude, and implications for qualifying usage regarding deficiencies relative to the criteria*, and iv) synthetizing a report on the review that overviews the effort, and integrates lessons learned. Results and discussion This exercise has been conducted in the given timeframe for the majority of the datasets. Challenges have been faced along the way, and among them : - *Agreeing on NDA* with data providers took more time than expected, due to countries' specific requirements; - *Need for sufficient information:* datasets have to be provided with detail of underlying unit processes, accompanied by the detailed description of what has been modelled and how; - The developed set of criteria for this exercise are not final review criteria for datasets: they are acknowledged as a starting point for further debates and improvements that should involve the broad LCA community. - Another question of interest raised during this exercise is the *confidentiality of the data*. Conclusions This hands-on exercise provided some valuable insights into the road towards global consensus and guidance on data review. Beyond this time-constrained effort, a broader discussion and consensus at international level on the review of datasets and a continuous effort in reviewing datasets is desirable. References [1] 2011. Global Guidance Principles for Life Cycle Assessement databases Paris, France. UNEP. 160 p.

Contaminants of Emerging Concern in the Environment and their Management (III)

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Investigating the Occurrence and the Fate of UV filters in Swimming Pools

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Chemical UV filters belong to emergent organic compounds that are turning out to be a sticking point for both environmental and health concerns. Today, these compounds are used in increasing quantities and can be found in various environmental media. However, little is known about the occurrence of UV filters in seawater swimming pools and about their reactivity in presence of disinfectants such as chlorine. Determining the concentrations of UV filters in swimming pools and their transformation products is paramount to any risk assessment consideration especially that their potential halogenated byproducts could be toxic. For this reason, samples from two seawater swimming pools located in Southeastern France were analyzed to determine the concentrations of 5 widely used UV filters, namely dioxybenzone, oxybenzone avobenzone, octocrylene, and 2-ethylhexyl-4-methoxycinnamate (OMC). Additionally, the reactivity of the UV filters in the presence of different molar ratios of chlorine in seawater was examined and their byproducts were identified through laboratory-setting experiments. Samples were treated using liquid-liquid extraction and analyses were performed using ultra performance liquid chromatography-quadrupole time-of-flight mass spectrometer (UPLC/Q-ToF-MS) and using gas chromatography coupled to an electron-capture detector (GC-ECD). Identification of byproducts was conducted by accurate mass measurements. MS-MS experiments were also performed to elucidate structures of the found compounds. This work showed that the levels of UV filters in swimming pool samples varied considerably from one compound to another and from a pool to another. Samples from the non-chlorinated pool contained more than one UV filter while in the chlorinated pool only octocrylene was detected. The reactivity/stability of the UV filters also varied depending on the compound's structure. While dioxybenzone and oxybenzone were found to be the most reactive with chlorine, octocrylene was stable and did not react with chlorine even in presence of high levels of the latter. These differences in reactivity with chlorine among the UV filters might explain the results of the occurrence of UV filters in the chlorinated pool samples with only the stable UV filter octocrylene being detected. Chlorination by-products of the reactive UV filters were identified for the first time in this study and transformation pathways were proposed based on the identified byproducts.

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Efficiency of reactive oxygen species in the degradation of the main synthetic musk compound: HHCB

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Synthetic musk compounds are the dominant artificial fragrance compounds used as substitutes for natural musks. Due to their extensive use in personal care and household products, they are produced at the tonnage scale, which placed them on the list of chemicals for priority action (OSPAR, 2000). Recently, synthetic musks have raised considerable attention due to their persistence in the environment and hazardous potential to ecosystems even at low concentrations. Wastewater treatment plants (WWTPs) represent the major route of musk contamination into the aquatic environment. It has been recognized that these substances are only partially degraded during treatment process, and little is known about their transformation products that should be included in all assessments concerning musks' fate and toxicity. The aim of this work was to investigate the occurrence of synthetic musk compounds and their degradation products in WWTPs and to evaluate their fate during treatment processes. Experimentally, new advanced oxidation processes have been also evaluated for the degradation of the main synthetic musk compound (HHCB,Galaxolide®). Several treatments combining oxidative and photochemical processes have been tested and the formation/degradation of products were investigated using time series experiments. The results have identified efficient degradation pathways for wastewater treatment and highlight the need to focus on degradation products and their potential toxicological effects.

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Comparison of UV/H2O2 and UV/S2O8 2- photolysis efficiency for the removal of estrogens in treated wastewater: chemical and biological assessment.
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Conventional Wastewater Treatment Plants (WTPs) are not designed to treat organic micropollutants and can only partially degrade them. Amongst hundreds of detected molecules, endocrine disruptors compounds (EDCs) are found at very low concentrations (in the ng/L range) but often sufficient to induce biological effects due to their high estrogenic potency. The natural estrogens, estrone (E1), 17β-estradiol (E2) and the synthetic one, 17α-ethinylestradiol (EE2) are the most active and commonly found estrogens in wastewater. In a near future, WTPs will have to be upgraded as they are a major cause of EDCs discharge in the environment. Previous studies pointed out that UV/H?O? process was a feasible way to remove estrogens from surface water and wastewater in terms of removal efficiency and energy consumption. However, attention has been recently paid to sulfate radical-based advanced oxidation process. Endocrine disruptors such as Bisphenol A were shown to be better degraded by UV/$S_2O_8^{2-}$ than UV/H?O? but no study have been conducted using UV/$S_2O_8^{2-}$ photolysis for the oxidative removal of E1, E2 or EE2. Therefore, in the present study, UV/H?O? and UV/$S_2O_8^{2-}$ photolysis using a commercial low pressure (λ=254nnm) reactor were compared for the removal of a mixture of three hormones (E1, E2 and EE2) under semi-real conditions. Global estrogenic activity and single compound degradations were measured at the same time. The formation of various by-products that could potentially be more toxic or estrogenic than the original compound were assessed by YES bioassay (estrogenic activity) and *Vibrio fischeri* bioassay (acute toxicity) for each treatment in treated wastewater. Treated wastewater was obtained from a local WTP (Feyssine, Lyon) and spiked with hormones at 5 μM. Hormones removal was measured by UHPLC-UV. UV in combination with H?O? or Persulfate led to a fast estrogenic activity removal. However, global estrogenic degradation constant was 2.43 times faster following UV/$S_2O_8^{2-}$ photolysis than UV/H?O? photolysis. Degradation constants of single compounds were between 2.34 (E1) and 3.9 (E2) faster following UV/$S_2O_8^{2-}$ treatment. Both treatment could enhance hormones and global estrogenic activity removal at the same time but UV/$S_2O_8^{2-}$ was more efficient at equimolarity. No estrogenic or toxic by-products were detected.

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Advantages of wastewater treatment plant upgrading with additional activated carbon for ecosystem health
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Aquatic organisms have to cope with a variety of anthropogenic substances continuously released into the aquatic environment. In order to reduce surface water pollution, new wastewater treatment plant technologies have been developed during the last years. However, little is known about their efficiency with respect to ecosystem health. The BMBF project SchussenAktiv*plus* examined the efficiency of different wastewater treatment technologies and the resulting advantages for the ecosystem. For this purpose, different chemical and biological analyses were conducted prior to and after the application of new technologies to several wastewater treatment plants and rainwater overflow basins connected to the Schussen River, a major tributary of Lake Constance. Another tributary, the Argen River, served as a reference. The present study is integrated into the follow-up project SchussenAktiv*plus*+ and focuses on the long-term effects of the additional activated charcoal stage at the wastewater treatment plant Langwiese (Ravensburg, Germany) for the ecosystem of the Schussen River, with special focus on fish health. In order to assess endocrine and toxic effects in fish, different biological analyses were conducted prior to and after the upgrade. Thus, the induction of vitellogenin, the induction of micronuclei in blood cells, EROD activity, and fish development were examined. Overall, the results imply a positive impact of the additional activated charcoal stage for the ecosystem of the Schussen River.

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Biological treatment of micropollutants in drinking water resources
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Contamination of drinking water resources is becoming a threat that is particularly widespread. Micropollutants are emerging substances in surface and groundwater causing contamination of drinking water resources and ultimately to closing down groundwater abstraction wells. BIOTREAT is a European project in which *sustainable biotechnologies* are developed for remediation of drinking water resources based on bioaugmentation, introduction of specific degrading microorganisms or microbial consortia into existing sand filters at waterworks or mobile filters. The compound BAM (2,6 Dichlorobenzamide) was chosen as model compound. Based on results of laboratory tests, a total of 6 submerged sandfilter columns were operated, of which 2 none-inoculated controls and two columns that were exposed to 1 μg/L BAM and two to 30 μg/L BAM (all inoculated with MSH1). Start-up retention time was 120 hours and the operation time of the experiment was 240 hours. No back-flushing was applied. Results show that in the 1 µg/L BAM exposed columns, BAM degradation up to 72% was established. In the 30 µg/L BAM exposed columns, 99% degradation was achieved. Well relocation is the cheapest scenario in case of pollution with micropollutants. But the well replacement scenario is only cheaper if the new well is placed within a few kilometers from the water production plant (3.5 kilometers for the 800,000 m^3 water production per year). If clean water is more than a few kilometers away, implementing a BAM removal strategy becomes the best solution. For the large drinking water production volumes a GAC filter is the cheapest BAM removal technique. BIOTREAT bio-augmentation technology + carrier is the most attractive technology for small scale drinking water production plants. Compared to traditional physicochemical technologies, biological technologies have the advantage that: - Natural resources are used. - No harmful chemicals are used, use of contaminated groundwater for drinking water production is made possible which otherwise might remain unused. - Application is low cost compared to traditional physicochemical technologies. - Technology is plug and play. No additional infrastructure needed at waterworks. - Compound specific removal is possible. Based on a production facility of 800.000 m^3 per year the operating costs for these technologies, bioaugmentation and bioaugmentation + carriers, are € 0.20/m^3 and € 0.11/m^3 respectively.

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Triclosan, triclocarban and parabens in greywater: identification of their sources.
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The use of soaps, shampoos or other personal care products (PCPs) generates discharges of chemicals into the environment through grey- and wastewater. Among these chemicals, parabens (PBs), triclosan (TCS) and triclocarban (TCC) are widely used in the composition of PCPs but also in sportive clothes, pharmaceuticals and food products as antiseptic or conservative. In a previous study several kinds of greywater from shower, washbasin, manual dishwashing, dishwasher and washing machine have been analyzed. The key lessons highlighted by this work were that (i) greywater strongly contributes to the contamination of wastewater and (ii) washbasins, showers and more surprisingly washing machines are the main contributors (in loads, μg/inhabitant/day) to the contamination of wastewater. PCPs are not therefore the only source of parabens and triclosan in wastewater: dishwashing and washing machine samples appeared as contaminated as shower samples. To reduce micropollutants at source, a better knowledge of the origin of greywater contamination is needed. The identification of the origin of the contamination was assessed through the decomposition of waters from washing machine (n=4) and shower (n=4) into four types of samples (1, 2, 3 and 4). Consequently, several potential sources were investigated: **washing machine:** tap water (1), washing machine (2), laundry products (3) and clothes (4); **shower:** tap water (1), bathtub (2), personal care products (3) and volunteer (4). First, whatever the compound, the tap water appears to be not or very slightly contaminated by TCC and PBs. In the same way, the contribution of the bathtub appears to be negligible. Concerning MeP, EtP and PrP, the main contributors are the products (55, 68 and 62 % respectively) and the body (39, 32 and 35 % respectively). For BzP and TCC, products are the only source of contamination. PBs and TCC are used as antiseptic or conservative in numerous PCPs, including toothpastes, soaps, deodorants and cosmetics products which led to the important contribution of products to the whole contamination. However, the striking contribution of the body was more surprising meaning that an important part of the contaminants (MeP, EtP and PrP) arises from the skin. Three hypothesis have been advanced to explain our results: after the use of PCPs, a part remains on the skin; a deposition on the skin of air dust containing PBs and TCC; and a transfer of contaminants from clothes to skin.

Ecotoxicology and risk assessment of nanomaterials - Interactions at nano-bio interface (I)

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Tracking nanomaterial targeting and uptake by SUSTU, protein corona surface proteomics
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The nanosafety of any newly developed nanomaterial should be studied through its evolution and aging upon interaction with environmental or biological samples rather than at the stage of newly synthesized entity. One of those key elements is the understanding of the nano-bio-eco interphase. The nature of the protein corona (PC) in contact with different biological environments has been extensively reported [1-3]. Nevertheless, less few studies addressed the evolution of the PC in terms of how the transition from an extra- to intra-cellular environment can modulate its biological identity and physicochemical properties [3, 4]. By understanding the key questions to be addressed from a new synthesized nanomaterial we can focus our analysis in the minimal set of data that would provide that information. Following this shortcut strategy we have developed, SUSTU, a surface proteomic method for tracking nanoparticle targeting, uptake and safety based on nanoparticle protein corona. The objective of this work are: i) develop methodologies that can define the specific enrichment of the PC surface, and iii) integrate MS-based PC data to develop nanointeraction tools for nanosafety. The method developed is highly reproducible between biological and experimental replicates. The key steps has been considered in the method development to be able to compare the data from the entire corona to the surface. By comparing the quantitative analysis of entire protein corona to the surface protein corona, we confirmed that the distribution of proteins in the surface is followed different pattern than in the entire corona. The protein concentrated in the surface are not equally distributed in the internal core of the corona. We could define a set of proteins that could be involved in targeting and other set that are not exposed to interaction with biological systems. We discussed the biophysical difference among the proteins composing the surface and the entire corona and the biological implication at the level of targeting and uptake capability and nanosafety at destiny.

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Algal toxicity of platinum nanoparticles - Implications of NP aggregation, dissolution and shading
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Platinum nanoparticles (PtNPs) are used in automotive catalytic converters and emitted to the environment during use. The resulting aquatic toxicity of PtNPs remains largely unknown. Algal growth rate inhibition has been reported, however, this endpoint is substantially influenced by PtNPs shading effects, and it is unclear if any toxic mechanism(s) are involved. Present study elucidates the mechanisms involved in the algal growth inhibition caused by PtNPs by comparing different toxicity endpoints in two algal species and media, including influence of NP shading, dissolution, aggregation, and adhesion to algal cells. Algae were exposed to $PtCl_4$ and 5.7 nm starch stabilized PtNPs in standard growth rate inhibition tests (ISO 8692:2004) with 48h incubation and a 2h C-assimilation test with the green algae *P. subcapitata* and *C. reinhardtii*. Shading effects were studied using a double-vial setup, containing algal cells in inner vials, surrounded by PtNPs in larger outer vials. After 2, 24 and 48h, oxidative stress and cell membrane damage were determined by flow cytometry and body burdens of PtNPs (Pt mass/cell) determined using a coulter counter and ICP-MS analysis of cells captured on filters. Characterization included size distribution (AsFlFFF, NTA, DLS), dissolution (ultracentrifugation), sedimentation (UV-VIS) and abiotic ROS generation (fluorescent dye, UV-VIS). PtNPs inhibited growth rates of the algae in the standard test and slightly less in the double-vial shading setup, suggesting that shading occurs but that PtNPs inhibits growth by other means than shading. In the 2h C-assimilation test, inhibition was similar using the standard or the double-vial setups, thus inhibition was solely ascribed to shading, making this test inappropriate for testing PtNP-toxicity. If PtNP concentration-response curves were based on dissolved fractions, higher toxicity was found than accounted for by the ionic Pt, suggesting a NP-specific effect. *P. subcapitata* was more sensitive to $PtCl_4$ and PtNPs and was shaded more, than *C. reinhardtii*, which correlates with a greater measured body burden of PtNPs in *P. subcapitata*, which again might be related to less PtNP aggregation in the ISO media. PtNPs caused extensive oxidative stress, but little membrane damage. *P. subcapitata* was less affected than *C. reinhardtii*, which is opposite to the growth rate inhibition tests. Thus, growth rate inhibition and oxidative stress do not appear linked in a straight forward manner.

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Mechanistic understanding toward the toxicity of graphene-family materials to freshwater algae
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In this work, we systematically investigated the toxicity of graphene-family materials (GFMs) including graphene oxide (GO), reduced graphene oxide (rGO) and multi-layer graphene (MG) to alga (*Chlorella pyrenoidosa*). GFMs exhibited much higher toxicity than other carbon materials (e.g., carbon nanotube and graphite), with the 96 h median effective concentration (EC_{50}) values of 37 (GO), 34 (rGO), and 62 (MG) mg/L. Heteroagglomeration between GFMs and algal cells contributed to algal growth inhibition. rGO and MG were easier to agglomerate with alga than GO. Cell membrane was damaged after GFMs exposure as indicated by the increased leakage of K^+ and DNA, and rGO caused the highest damage. Oxidative stress and physical penetration were responsible for the observed membrane breakage. For GO, shading effect contributed approximately 17% of growth inhibition due to its higher dispersibility while the other GFMs did not show any shading effect. All the three GFMs adsorbed macronutrients (N, P, Mg, and Ca) from the algal medium, leading to nutrition depletion and indirect toxicity. For GO, rGO, and MG, the contribution of nutrition depletion to growth inhibition was 53%, 35%, and 27%, respectively. The information provided in this work will be useful for understanding the environmental risk of graphene materials in aquatic environments.

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Internalization and toxicological mechanisms of uncoated and PVP-coated cerium oxide nanoparticles in the freshwater alga Chlamydomonas reinhardtii
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Cerium oxide nanoparticles (CNPs, nanoceria) are increasingly used in industrial applications and may be released to the aquatic environment, where the exposure of aquatic organisms becomes likely. There are contradictory reports on whether nanoceria may act as an oxidant causing toxicity[1] or as an antioxidant being able to scavenge free radicals[2], given that the toxicological behaviour of these nanoparticles is still poorly understood. Moreover, little is known about the internalization process of CNPs in algae. There is evidence of CNP-internalization by *Chlamydomonas reinhardtii* (*C. reinhardtii*), but the internalization mechanism and route of uptake are still unknown[3]. In this study, we used an uncoated and different polyvinylpyrrolidone-coated CNPs (the purpose of the coating being to improve their stability, by inhibiting aggregation) with the aim of identifying their internalization and toxicological mechanisms. Monodispersed nanoparticles were synthesized and physicochemically characterized both in distilled water and the exposure media. Nanoparticles coated with PVP, irrespective of PVP molecular weight, had higher growth inhibition to *C. reinhardtii* than bare CNP. PVP-CNPs significantly increased ROS formation in exposed cells, indicating that oxidative stress might be an important toxicity mechanism. Interestingly, there was evidence of membrane disruption upon nanoparticle exposure, suggesting that membrane permeability might increase; thus, allowing internalization of nanoparticle. At present, the mechanisms of CNP-internalization are under thorough study. 1. Pulido-Reyes G, Rodea-Palomares I, Das S, Sakthivel TS, Leganes F, Rosal R et al. Untangling the biological effects of cerium oxide nanoparticles: the role of surface valence states. Scientific Reports. 2015;5:15613. doi:10.1038/srep15613. 2. Das M, Patil S, Bhargava N, Kang J-F, Riedel LM, Seal S et al. Auto-catalytic ceria nanoparticles offer neuroprotection to adult rat spinal cord neurons. Biomaterials. 2007;28(10):1918-25. 3. Taylor NS, Merrifield R, Williams TD, Chipman JK, Lead JR, Viant MR. Molecular toxicity of cerium oxide nanoparticles to the freshwater alga Chlamydomonas reinhardtii is associated with supra-environmental exposure concentrations. Nanotoxicology. 2015:1-10. doi:10.3109/17435390.2014.1002868. *Acknowledgement* - This research was supported by CTM2013-45775-C2-1-R and CTM2013-45775-C2-2-R grants from MINECO.

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Silver nanoparticles vs. dissolved silver toxicity to Daphnia magna: Unique modes of action?
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Disturbances in genes or biochemical regulation of an organism can often be seen at lower doses or on shorter time scales than whole body effects, and are a

precursor for whole body manifestations of a toxicant and population level effects therein, and thus are detected sooner than such endpoints as immobility. If we are able to predict effects prior to whole body manifestations through more sensitive screening we are able to better protect the environment. For nanoparticles (NPs) the best biochemical endpoints, and those most relevant to highlight exposure to NPs, still remain elusive. For instance, it is still uncertain if silver NPs (Ag NPs) have a distinct mode of action (MOA) from dissolved Ag (d-Ag) within the aquatic environment; this is despite a large body of research. d-Ag acts at the Na^+K^+-ATPase ligand, causing osmoregulatory failure and mortality mainly through an imbalance of sodium and potassium. Many NP studies show that oxidative stress may be the main driver of NP toxicity. Contradictory research often arises from researcher postulation based on standard test results (i.e. mortality/ reproductive tests) coupled with interpretation of the NP physicochemical characteristics with no evidence at the biochemical level of the interaction at the biophysical interface, or indeed if there is a common interaction interface between the two forms. Here we used a series of biochemical tests to identify differences and/or similarities between Ag NP and d-Ag toxicity and their MOA, in particular we measure whole body cation levels to see if distinct MOA were present from that traditionally noted for d-Ag. We also assess if the degree of effect correlates with the degree of effect seen for 3 different Ag NPs and dissolved silver (as $AgNO_3$) in acute bioassays. d-Ag caused physiological perturbations in cation levels as previously noted in the literature. Ag NPs affected mitochondrial function which led to perturbations in ATP and increased super oxide with a concurrent rise in super oxide dismutase in cellular defence. The degree of effect for immobility and biochemical changes were similar in some instances. Based on the results we present a flow chart of possible physiological reactions which lead to eventual population impacts. We discuss the possibility of a common MOA across many species based on the current state of knowledge for Ag NPs. Such knowledge may lead to more specific and better testing strategies for Ag NPs.

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Development of a multimodal imaging approach of Ag-NP interactions with Raphidocelis subcapitata

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Abstract Nanotechnology has seen tremendous advances over the last few decades and has found applications in a diverse spectrum of industries, ranging from cosmetics, medical diagnostic imaging, and forensics, to energy and catalysis. These applications exploit the unique properties that arise when materials are manufactured at the extremely small size that is referred to as 'nanoscale'. Importantly, it is now recognised that due to their small size and high surface area, engineered nanomaterials (ENM) may interact in a distinct manner with living organisms in the environment. However, techniques that are capable of elucidating the interactions of ENMs with livings systems (the bio-nano interactions) are currently critically limited and are in high demand [1]. This is a serious research challenge given the inevitability of ENMs release into the environment by the development and use of ENM-containing products. The aim of this study was to develop a methodology enabling imaging of bio-nano interactions between silver nanoparticles (Ag-NPs) and the algae *Raphidocelis subcapitata*, a model organism in toxicity studies. Of the thousands of ENMs that have been identified in the consumer market [2], Ag-NPs dominate in their occurrence and are hence the most likely ENMs to enter the environment. Silver is known to be toxic towards a variety of organisms, and for this reason their risk assessment has been of particular focus in many studies. We used a multimodal approach combining dark-field light microscopy, scanning electron microscopy (SEM) and nanoscale secondary-ion mass spectrometry (NanoSIMS) imaging to investigate the interaction of surface functionalised Ag-NPs with the *R. subcapitata* cells. By using the dark-field light microscope and high resolution SEM, we identified the presence of Ag-NPs in the peripheral of the algae, and confirmed its chemical identity using NanoSIMS imaging. The advantages and limitations identified for each technique in determining the localisation of NPs in single organisms will be presented and directions for future improvements in this combined approach will also be discussed. **References** [1] Schaumann, G. E.; Philippe, A. et al., 2015 Understanding the fate and biological effects of Ag- and TiO(2)-nanoparticles in the environment: The quest for advanced analytics and interdisciplinary concepts. Sci Total Environ**,** 535: 3-19.\n [2] Forbrugerrådet Tænk The Nanodatabase. http://nanodb.dk/en/, Accessed [24/11/2015].

Flame Retardants: Alternatives, Environmental Fate and Toxicity (II)

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From clothing to laundry water: Investigating the fate of flame retardants and plasticizers sorbed to fabrics.

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Our indoor environment contains large inventory of products that contain semi-volatile chemicals of concern such as flame retardants and plasticizers. Over time, these chemicals, which are added but not chemically bonded to polymers, are released from those products and accumulate in the various media within the indoor environment such as dust and fabrics. We report on first, the partitioning from air-to-fabric and then the loss of chemicals sorbed onto fabrics through laundering. Analytes of interest were organophosphorus (OPFRs) and brominated flame retardants (BFRs) and phthalates. Cotton and polyester fabrics were deployed in an office at University of Toronto for 30 days followed by their laundering and analysis by GC-MS to compare total mass sorbed, mass released in laundry water, and mass remaining on fabrics after laundering and drying. Results showed that fabrics accumulated all analytes tested. More than >70% of sorbed OPFRs, such as TCEP and TCPP, were lost from fabric to laundry water whereas this value was < 10% for BFRs. Rather, BFRs remained sorbed to fabrics after laundry and drying. These results are interpreted in terms of chemical fate indoors and outdoors. The results can explain, in part, the source of elevated concentrations of OPFRs in waste water streams and eventually receiving surface water bodies (e.g., Schreder et al. 2014). Thus, our clothing (or fabrics) can act as a pathway for ecosystem exposure and/or transfer of COCs from indoors to outdoors.

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Organophosphate and Brominated Flame Retardants in Human Hair and Nails

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After the phase-out of polybrominated diphenyl ethers (PBDEs), the use of alternative flame retardants (AFRs), such as FireMaster 550, and of organophosphate esters (OPEs), has increased. However, little is known about human exposure to these chemicals. This lack of biomonitoring studies is partially due to the absence of reliable non-invasive biomarkers of exposure. Human hair and nails can provide integrated exposure measurements, and as such, these matrices can potentially be used as non-invasive biomarkers of exposure to these flame retardants. Paired human hair, fingernail, toenail, and serum samples obtained from 50 adult participants recruited at Indiana University Bloomington were analyzed by gas chromatographic mass spectrometry for 36 PBDEs, 9 AFRs, and 12 OPEs. BDE-47, BDE-99, 2-ethylhexyl-2,3,4,5-tetrabromobenzoate (TBB), di(2-ethylhexyl) tetrabromophthalate (TBPH), *tris*(1,3-dichloro-2-propyl)phosphate (TDCIPP), and triphenyl phosphate (TPHP) were the most abundant compounds detected in almost all hair, fingernail, and toenail samples. The concentrations followed the order: OPEs > TBB+TBPH > Σpenta-BDE. PBDE levels in the hair and nail samples were significantly correlated with their levels in serum ($P < 0.05$), suggesting that human hair and nails can be used as biomarkers to assess human exposure to PBDEs.

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Impurities of resorcinol bis(diphenyl phosphate) in plastics and in dust

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Flame retardants are added to a variety of materials (electronic equipment, textile, furniture, etc.) in order to prevent quick combustion and to delay the spread of fire. After the phase-out of polybrominated diphenyl ethers (PBDEs), phosphorus flame retardants (PFRs) have been increasingly used as suitable alternatives. However, concern about their widespread presence and potential toxicity has also increased in the last years.Resorcinol bis-(diphenylphosphate) (RDP) is a PFR widely used in electric and electronic equipment and ubiquitous in house dust according to recent literature. Similar to other flame retardants, RDP formulations and products treated with RDP can contain impurities, byproducts and breakdown products that could influence the total toxicity of RDP formulations. In this study, we investigate the presence of RDP impurities in plastics from electrical/electronic equipment and in indoor dust collected on electronics, in order to study the possible migration of these compounds into the environment. A variety of RDP-related products, such as *meta*-HO-triphenyl phosphate (*meta*-HO-TPHP), RDP with the loss of a phenyl ring (RDP-[Phe]) and meta-HO-RDP were observed in both plastic and dust samples collected on/around electronics. Regarding the dust samples (n=30), the detection frequency of the compounds were in the order TPHP (n=30), RDP (n=27), meta-HO-TPHP (n=25), RDP-[Phe] (n=8) and meta-HO-RDP (n=5). The concentrations measured in dust for the three compounds for which standards were available (222-50,728 ng/g for TPHP, 23-29,118 ng/g for RDP and 20-14,227 for meta-HO-TPHP) are in agreement with those previously reported for RDP and TPHP and for other FRs in samples collected on/around electronics . The high levels of RDP, TPHP and meta-HO-TPHP (reaching the ӝg/g levels) that were found in some of the dust collected on electronics suggest that dust could be a significant route of human exposure to these compounds via ingestion/dermal adsorption. This could happen especially when touching these surfaces, e.g. switching on a TV or a router. Giving these first results, more data on the presence and potential toxicity of meta-HO-TPHP (and of other RDP impurities) is necessary in future monitoring studies to assess the human exposure and risks of RDP related compounds. Besides, due to its specificity and ubiquity as RDP impurity, meta-HO-TPHP could be a suitable marker/tracer of RDP

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Toxicological mechanisms of current flame retardants
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Flame retardants (FRs) are chemicals produced at high volume that are introduced to nearly all manufactured materials with the purpose to limit fire hazard. Given their persistence and potential to bioaccumulate, the compounds have been found in serum and breast milk. Toxicological data is largely limited to monitoring classical endpoints such as measuring lethal dose values. Therefore there is little insight into their molecular modes of action that may give rise to their toxic phenotypes. Additionally, some previously-used FRs have been shown to elicit a wide range of toxicological effects and have thus been banned from use. Given the structural similarity to their toxic predecessors, their persistence, bioaccumulation, and lack of insight of toxicological and molecular mechanisms, currently-used FRs pose a significant risk. We therefore used a bacterial gene profiling assay to screen 12 currently-used FRs as to obtain mechanistic insights of toxicity. Bacterial biosensors are frequently used to assess ecotoxicological impacts of compounds since they are particularly useful in compound screening and classification according to mode of action. The assay consists of 12 bacterial reporters (*Escherichia coli*) responsive to oxidative stress, protein degradation, DNA damage, membrane damage, and growth arrest. Both brominated and organophosphate FRs were tested. Nearly all compounds showed significant inductions in a majority of stress genes when compared to control treatments. When observing only raw absolute induction levels, the stress genes *ClpB*, *RecA*, and *MicF* were the only genes exhibiting fold inductions of greater than 2, indicating that these compounds result primarily in protein, DNA, and membrane degradation. However, accounting for the different induction potentials for each stress promoter and clustering using hierarchical and k-means algorithms, clusters corresponding to growth arrest and oxidative damage were also observed. This indicates that FRs may result in a loss of protein, DNA, and membrane integrity with generation of reactive oxidative species as the potential underlying mechanism, in agreement with other studies. Finally, the lack of any notable gene induction following DOPO treatment even at extremely high concentrations, along with its excellent fire-retardation supports its increasing interest as an alternative to halogenated FRs.

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Species differences in steroid hormone receptor responses
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Endocrine disrupting chemicals present in the environment can have differences in efficacy and potency when interacting with steroid hormone receptors from different species. This is due to both species variations in sequence and endogenous hormone utilization. There are a number of brominated flame retardants (BFRs) for which there are no information on their interactions with biological systems. We have been characterising BFRs using a combination of *in silico* molecular modelling, in vitro assays and in vivo studies. In an *in silico* analysis of 10 model BFRs we identified one of the test compounds, 1,2-dibromo-4 (1,2 dibromomethyl) cyclohexane (TBECH) as a partial androgen receptor (AR) agonist. TBECH is an interesting compound as it has 4 different diastereomers (α, β, γ, δ) each with its own enantiomer. In a follow up study we compared the efficacy and potency of the TBECH diastereomers and discovered that TBECH-γδ exhibited the same efficacy and potency as the endogenous androgen, DHT, while TBECH- αβ induced AR to about 30% of maximal activity. Continued screening using in silico methodology has resulted in the discovery of yet another group of BFRs that interact with the human AR. These are 2,4,6-tribromophenyl ether (ATE) and 2,3-dibromopropyl 2,4,6-tribromophenyl ether (DPTE) and the metabolite 2-bromoallyl 2,4,6-tribromopheyl ether (BATE) that we recently showed to be potent AR antagonists. As different species use different androgens we then focused on determining the ability of these novel BFRs to interact and regulate the activity of AR in different species as well as to determine if they interact with other steroid hormone receptors in a species-specific manner. TBECH was found to activate both the zebrafish AR and the chicken. However, the activation potency was lower than that observed for the human AR. TBECH-γδ induced a 40% increase in zebrafish and only about 15% in chicken. TBECH- αβ, that showed about 30% induction in human AR showed minimal induction of zebrafish and chicken AR at the maximum TBECH concentration. ATE, BATE and DPTE were equally potent at inhibiting AR signalling in human, zebrafish and chicken. Following the identification of these BFRs as AR agonists/antagonists we also used *in silico* modelling and analysis of transcriptional regulation and observed that these compounds also regulate other steroid hormone receptor pathways, including the oestrogen receptor and thyroid receptor signalling pathways.

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Determination of the eco-toxicological risk of eluates of reactive flame retardants
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Flame retardants are increasingly used in consumer products as e.g. electronics and furniture as well as in construction. Along with the increased usage, concerns about negative effects of flame retardants on human health and the environment have been raised. Concerning construction products one application for flame retardants is the furnishing of steel structures with reactive flameproof coatings. These coatings consist of a complex mixture of different chemicals, additives and solvents. For the evaluation of the risk possibly posed by their application to the environment, this whole mixture should be tested. Two different leaching tests were applied according to DIN/TS 16637-2 (sample permantly submerged in water) and DIN EN 16105 (immersion and drying cycles), respectively, which can be used for plate-like products. Eluates were divided into subsamples for the measurement of different parameters: pH-value, conductivity, total organic carbon (TOC), cation and anion concentration. For screening of organic compounds, aqueous samples were liquid/liquid extracted using three solvents with different polarity (toluene, hexane and ethylacetate). GC-MS chromatograms were recorded in the SCAN mode and mass spectra of the peaks were compared with spectras stored in the NIST library. Eluates of leaching test accroding to DIN CEN TS 16637-2 showed low electrical conductivity (20 to 150 µS/cm) and low release of cation and anions often near the LOQ. Diffferences could be observed for plates treated with the reactive coating at both sides and plates coated only at one side, while the other side is only furnished by the corrosive protection layer. Release of TOC and Zn was approx twice or 10 fold higher in the case of plates coated on one side. Furthermore, the comparison of the results for both types of plates showed that a large portion of the released compounds originate rather from the corrosion protection layer than from the reactive coating itself. Leaching tests according to DIN EN 16105 have not been finished yet, but first results show that the conductivity is much lower (1-4 µS/cm) than for the tests according to DIN/TS 16637-2 leading to the assumption that the release of compounds is lower. By comparison of the mass spectra with library data, mainly organic solvents as xylene, 1-methoxy-2-propanol acetate and n-btuyl carbamate have been tentatively identified so far in the first test fraction done by DIN CEN TS 16637-2.

Microplastics in the environment: Sources, Fate and Effects (III)

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Interactions of non-polar organic compounds with polyethylene and polystyrene micro particles
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Concerns about the presence of micro scale polymers (i.e., microplastics) in environmental system are rapidly growing. Understanding the interactions between microplastics and other contaminants is thus essential for evaluating the materials' fate. Additionally, sorption of organic compounds by microplastics is one of the major processes as it may affect the compound distribution in sediments and aqueous phases. However, there is currently little data on the sorption potential of diverse sets of hydrophobic organic compounds/microplastics systems that may occur in the environment. We applied a multi-phase sorption batch method to investigate the sorption of seven organic compounds by two most commonly found microplastics. Experimental sorption isotherm data by polyethylene (PE) and polystyrene (PS) measured over several orders of magnitude were fit to the non-linear Freundlich model to support mechanistic interpretations of the results. Correlation between sorption coefficients by microplastics and sorbates hydrophobicity indicates that hydrophobic interactions are of major importance. Linear isotherms by PE shows that uptake was driven by adsorption into the bulk polymer, while adsorption onto the surface seems to be the dominant sorption mode indicated by non-linear isotherm data for PS. However, the results emphasize that neither sorbate nor sorbent-specific parameters sufficiently explain the observed difference in sorption alone.

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Chemical Adsorption of Hydrophobic Polycyclic Aromatic Hydrocarbons in the Marine Environment onto Microplastic Polymers and Subsequent

Desorption in a Simulated Gut

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With an approximate degradation time of 450 years, plastic is one of the most durable and persistent environmental contaminants in the modern world. Production since the 1950s has increased prodigiously. In the marine environment, photo-oxidative degradation and the abrasive action of waves progressively degrade larger pieces of plastic into tiny polymer particles less than 5 millimetres in diameter, termed microplastics. As a consequence of their small size, microplastics are inadvertently ingested by ocean dwelling biota, particularly susceptible filter feeders such as Mytilus edulis and many species of fish. This can result in detrimental effects such as inhibition of gastrointestinal function and feeding impairment. However, it has emerged fairly recently that microplastic polymers collected from the marine environment have been found to have Hydrophobic organic chemicals (HOCs), such as polycyclic aromatic hydrocarbons (PAHs), adsorbed onto their surface. Since marine organisms ingest microplastics, it can be proposed that microplastics have the potential to act as a transporter for the conveyance of hydrophobic persistent organic pollutants (POPs), such as PAHs, into marine biota. PAHs are formed from incomplete or inefficient combustion of organic material, diagenesis and biosynthesis. While there is a consistent background level from forest fires and volcanic activity, a significant fraction of PAHs present in the environment is due to anthropogenic activities, such as internal combustion engines. Consequently, PAHs reach the marine environment via sewage and industrial discharges, oil spillages and deposition from the atmosphere. One particular characteristic of PAHs is their susceptibility to ultra-violet light. However, pericondensed PAH structures are more centrally condensed allowing them to withstand higher ultraviolet fluxes. This results in a decrease in susceptibility to photodecomposition by ultraviolet light and thus, resistance to degradation in the marine environment. Consequently, microplastics can then adsorb these contaminants. Since many of the susceptible organisms that ingest microplastics are located at the bottom of the food chain, chemical adsorption provides a potential mechanism for the bioaccumulation of contaminants in marine organisms and the potential trophic transfer of these contaminants up the food web to larger organisms, and possibly humans. Consequently, it is necessary to investigate this potential mechanism. \n

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Dioxin-like activity of compounds sorbed to 4 different plastic polymers deployed in the San Diego Bay area

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In the early 1970s plastic particles were detected in the marine system for the first time and by the late 1990s over 180 species were reported to ingest plastic particles. The occurrence of plastic litter in the marine environment poses not only a physical threat to organisms via entanglement and ingestion, but also a toxicological threat to wildlife. In recent years it has been demonstrated that various anthropogenic environmental pollutants can be found on different types of marine plastic debris. Most of these substances are considered as harmful to humans and wildlife. They can act as mutagens, cancerogens and endocrine disruptors and exert adverse health effects to organisms. The toxicological potential of anthropogenic pollutants derived from marine plastic is a yet little studied research area. In this study virgin plastic pellets of four different polymer types, polypropylene (PP), polyvinylchloride (PVC), polyethylene terephthalate (PET), and low-density polyethylene (LDPE) were deployed for nine and twelve months in different sites of the San Diego Bay area. The plastic pellets were extracted using hexane and ultra-sonication. Extracts were analyzed for their dioxin-like activity in the H4IIE luc assay, a mechanism specific bioassay based on a recombinant receptor/reporter vertebrate cell line, and chemical analysis of 23 PAHs including the 16 priority PAHs listed by the U.S. EPA. Bio-TEQs$_{25}$ and Chem-TEQs$_{25}$ values were calculated. Among the different polymer types LDPE demonstrated the highest dioxin-like activity in the samples followed by PP, PVC and PET. The calculated Chem-Teq$_{25}$ values supported a higher toxicity potential for LDPE compared to the other polymers. Studies of time dependent sorption were hampered by too few sampling time points but a slightly higher PAH concentration after twelve months compared to nine months could be observed indicating continuing sorption capacity. In contrary, the biological activity decreased from nine to twelve months. This leads to the assumption that other compounds than the analyzed PAHs might show a desorption from the plastic between 9 and 12 months which results in a decreased activity in the bioassay. This study shows that virgin polymer pellets are able to sorb pollutants from ambient water that induces a dioxin-like effect and that LDPE has a higher sorption capacity for PAHs and other dioxin-like compounds that were not measured in this study compared to the other polymers.

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Evaluation of the analysis of microplastics by mass spectrometry and assessment of their adsorption capacity for organic contaminants

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Plastic residues in the sea are an emerging environmental problem because of human activity. The presence of these marine debris particles in the ocean represents not only an aesthetic problem since they also can imply harmful damages for the coastal areas and the socioeconomic activities linked to these regions[1,2]. The low degradability of these plastics and their size hamper the oxygen exchange and light which lead to eutrofisation processes[2]. Among these marine debris, microplastics are risky for primary producers and their impact has not been fully evaluated [1]. Nonetheless, different studies revealed their ubiquitous presence in the sea as well as its uptake by marine biota[3]. MPs can enter into marine food web and, finally, into human diet. In addition, these MPs can accumulate persistent organic contaminants and pathogens being also an environmental distributor of these ones [1,4,5]. The main objectives of this work are the evaluation of different ionization sources coupled to mass spectrometry analyzers for the analysis of MPs (High Density Polyethylene, polystyrene and functionalized polystyrene-COOH) in water columns and; the evaluation of adsorption mechanisms of organic contaminants (*i.e.* polycyclic aromatic hydrocarbons (PAHs)) and emerging organic contaminants (*i.e.* perfluoroalkyl substances (PFASs)) onto MPs surface. The study of MPs by means of MALDI-TOF and APCI-QExactive allowed the chemical characterization as well as semi-quantitative analysis in the case of the last one. These technologies can be applied in the future for the analysis of environmental matrices such as water columns, sediments or biota. Regarding adsorption experiments under a controlled sea water conditions, the results evidenced that MPs can behave as carriers of the studied PAHs and PFASs in marine environments. The compounds are adsorbed onto MPs surface after short exposure time and these can act as carrier materials being an important contamination source of selected organic contaminants for marine flora and fauna. **Keywords:** microplastics, mass spectrometry, carrier materials of organic contaminants [1] Cole M. et al. Marine Pollution Bulletin 62 (2011) 2588–2597. [2] Harrison J.P. et al. Marine Technology Society Journal 45 (2011) 12-20. [3] Cole M. et al. Environmental Science & Technology 47 (2013) 6646-55. [4] Llorca M. et al. Marine Pollution Bulletin 87 (2014) 286–291. [5] Riosa L.M. et al. Marine Pollution Bulletin 54 (2007) 1230–1237.

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Characterisation, Quantity and Sorptive Properties of Microplastics Extracted From Cosmetics

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Cosmetic products, such as facial scrubs, have been identified as potentially important primary sources of microplastics to the marine environment. To better understand the source, we characterised, quantified and then investigated the sorptive properties of plastic microbeads that are used as exfoliants in cosmetics. Polyethylene microbeads were extracted from several products, and shown to have a wide size range (mean diameters between 164 to 327 µm). We estimated that between 4594 – 94500 microbeads could be released in a single use. To examine the potential for microbeads to accumulate and transport chemicals they were exposed to a binary mixture of ^{3}H-phenanthrene and ^{14}C-DDT in seawater. The potential for transport of sorbed chemicals by microbeads was broadly similar to that of polythene (PE) particles used in previous sorption studies. In conclusion, cosmetic exfoliants are a potentially important, yet preventable source of microplastic contamination in the marine environment.

Cost effective and ecological relevant approaches in environmental toxicology using invertebrate species

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Is Multixenobiotic Resistance System in Daphnia magna a general stress response mechanism?

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Recent investigations show that the cellular multixenobiotic resistance (MXR) system represents a broad-scale defense mechanism protecting cells against environmental toxicants. The system is mediated by membrane transporter proteins which pump chemicals out of the cell, thus keeping their levels low. Organisms and cells with potent MXR defense are less sensitive to the toxic impact of compounds, because comparatively few of those molecules reach their site of toxic action. In a recent study this system was characterized in the crustacean *Daphnia magna*, sequencing four gene transporters that were constitutively expressed across live stages and induced by several chemical contaminants. Transporter activirty of this proteins were also characterized showing that inhibition of MXR-like efflux activity by MXR-inhibitors co-administered with toxic substrates of MXR enhanced the toxicity of the latter compounds. However, model ABC transporter substrates, inducers and inhibitors used did not allow distinguishing between ABCB and ABCC transporters activity types. Here, we present results of experiments designed to clarify the role of these transporters and its specificity. We selected tolerant clones by exposing outbreed populations to high concentrations of chemicals that are known to be substrates of MXR. Selected tolerant clones were then used to characterize MXR mechanisms. The response of heat shock proteins (Hsp70) was also studied to distinguish between substrate specific MXR responses from general stress. An ABCB knock-down gene clonal line was also developed taking advantage of the recent development of the CRISPR/Cas9 methodology in Daphnia. Results showed successful selection of tolerant clones relative to the lab clone by mitoxantrone, pentachlorophenol and ivermectin. Mitoxantrone and pentachlorophenol enhanced the transporter activity in their respective selected tolerant clones. However, high transcriptional levels of the abcb1 gene were only positively related with high transporter activity in the selected pentachlorophenol tolerant clone. All tolerant clones showed high constitutive levels of Hsp70 protein, which means that the selection of clones with enhanced levels of MXR may be part of a general stress response. In conclusion tolerant genotypes having higher levels of general stress and MXR proteins exist in natural populations of *D. magna* and can be selected by toxic compounds that are substrates of ABC transporters.

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Functional characterization of hemocyte populations of zebra mussel, Dreissena polymorpha and use in acute ex vivo toxicity tests

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The zebra mussel, *Dreissena polymorpha,* is an invasive freshwater bivalve mollusk native from Ponto-Caspian region that successfully colonized Western Europe and North-America. Because of its biological characteristics, transplanted or natural populations of zebra mussels were used as sentinel species in freshwater pollution monitoring and also in laboratory studies. Immune system constitutes a central point in organism homeostasis. This physiological process relies on hemocyte activities that are sensitive to chemical stress. Disturbance in immune response may affect mussels in their related populations. The first objective of this study was to characterize *D.polymorpha* hemocyte subpopulations under natural conditions. According to microscopy and flow cytometry analysis, three types of circulating hemocytes can be observed: hyalinocytes and blast-like cells for agranular hemocytes and one granulocyte population. Flow cytometry analysis of hemocytes functionalities indicated that blast-like cells possessed low activities and that hyalinocytes and granulocytes are fully equipped to perform innate immune response and exhibited close functionalities. The second objective of this study was to assess the cytotoxic effects of cadmium, a non-essential metal and persistent pollutant, on hemocyte subpopulations by involving a multi-marker approach. Hemocytes were exposed *ex vivo* to concentrations of cadmium ranging from 10^{-6}M to 10^{-3}M for 21 hours prior flow cytometric analysis of cellular markers. Measured parameters (viability, phagocytosis, oxidative activity, lysosomal content and mitochondrial activity) were affected in a dose-dependent manner. Analysis indicated different sensitivity to cadmium depending on cell endpoint and cell subtype. This work highlighted importance of hemocyte characterization for a better understanding of toxicological responses of invertebrates to xenobiotics.

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Highly cost-efficient determination of efflux pump inhibition and chemosensitization using single embryos of the pond snail Lymnaea stagnalis

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Measurements of efflux pump activity in single embryoes of *Lymnaea stagnalis* was shown to be a highly cost-efficient alternative to systems using human cell cultures. Results for the inhibitory effects of a range of pharmaceuticals showed the same effects in the same concentration ranges as previously reported in cell culture experiments. Further, the system was shown to be able to distinguish different modes of inhibition as well as being able to conveniently show chemosensitizing effects of pharmaceuticals.

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Time-course immunomarker assessments and life-history traits in the freshwater snail Lymnaea stagnalis in response to environmental contaminants

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Animals are under constant **multistress** pressures (e.g. chemicals and microbes). Hence, their immune system may be affected by the presence of chemicals (i.e. immunotoxicity). As a result, internal defences are inoperant to remove the microbiological burdens with possible consequences at the population level. In ecotoxicology, development and comprehension of biological tools that evaluate the effects of chemicals onto the **immune system** of invertebrate species are required (Galloway and Depledge 2001). *Lymnaea stagnalis* is a freshwater **gastropod** ecologically representative of lentic systems from holarctic zones of the planete. This species has been promotted in OECD guidelines for reprotoxicity testings (Ducrot 2014). It has the particularity to extrude a sufficient quantity of hemolymph that allows an **individual approach** (i.e. not pooled) of immunomarkers assessment in an **non invasive** manner, which also means that a **time-course evaluation** can be realized withtout animal sacrifice. Plus, hemolymph can be collected easily only by using a micropipette. Gust et al. (2013) reported immunotoxicity of contaminated surface waters toward *L. stagnalis*. More investigation remains essential in order to understand the feasability of using markers of immunotoxicity from *L. stagnalis*. Herein we investigated the time-course evolution of individual snails **hemocyte biomarkers** (i.e. density, viability, oxidative activity, phenol-oxidase activity and activity of phagocytosis) under contamination contexts. Simutaneously, an evaluation of **life-history traits** was carried out (i.e. survival, reproduction and feeding behavior). **The pyrethrinoid Deltamethrin** was set up in a flow-through system. The **Cadmium chlorure** ($CdCl_2$) was set up in semi-static conditions. Hemocyte biomarkers were evaluated by flow cytometry (densities, viabilities and activity of phagocytosis) and by spectrofluorimetry (phenol-oxidase and NADPH-oxidative activities). Reproduction was followed by collection of egg-masses and observation under binocular microscope. We observed that phenomenons of immunotoxicity may be transient (i.e. biological recovery) and so the time-course tracking of individual parameters is important. The comprehension of hemocyte biomarker variability is mandatory : inter-populations, intrapopulation (i.e. inter-individual) and intra-individual (i.e. time effect, physiological fluctuations). Thus, *L. stagnalis* deserves more attention as model organism in immunotoxicology.

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Nickel toxicity to tropical marine biota

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Over two-thirds of the world's nickel production is in tropical regions including Indonesia, the Philippines, Australia, New Caledonia, Brazil and Cuba. The mining, refining and smelting of lateritic ores can present many potential environmental risks, as with any such operations located in tropical regions of high ecological diversity. However, our current understanding of the toxicity of nickel to tropical biota is limited, largely due to the lack of ecotoxicity data with tropical species endemic to these regions. The Nickel Producers Environmental Research Association is currently leading a research program to develop and apply risk assessment tools to support the sustainable development of lateritic nickel deposits in the tropical region of South East Asia and Melanesia (SEAM). This research involves the compilation of exposure and effects assessment data for nickel in tropical marine waters, specifically in the SEAM region. Key gaps for ecologically important tropical taxa of the SEAM region include corals, crustaceans, gastropods, bivalves and fish. The aim of this study was to investigate the sensitivity of tropical marine biota to nickel, with a focus on those species relevant to the SEAM region. Data produced from this study will contribute to the development of a reliable and ecologically relevant water quality guideline value for nickel in tropical marine waters. Chronic nickel toxicity to two crustaceans (copepod and barnacle) and one gastropod (snail) native to the SEAM region was investigated. Toxicity tests were carried out at temperatures of 28-30 C and a salinity of 35‰. Endpoints measured included 72-h development (from egg to copepodite), 96-h metamorphosis and 96-h growth for the copepod, barnacle and snail, respectively. Throughout all tests, water quality parameters were monitored and sub-samples were taken to measure total and dissolved ($< 0.45\ \mu$m) nickel. The copepod was the most sensitive to nickel, followed by the barnacle and snail, with IC10 (10% inhibition concentration) values of 15, 72 and 79 μg Ni/L. This study provides valuable toxicity data which can be used in the development of an ecologically relevant guideline value for nickel in tropical marine waters.

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Effects of multiple stressors on freshwater macroinvertebrates in Iberian rivers

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Aquatic ecosystems worldvide are impacted by a variety of stressors, including organic and inorganic pollution, excess input of nutrients, geomorphological alterations, land use changes, hydrological stress, invasive species and pathogens

[1]. As a consequence, the biodiversity decline is one of the greatest ecological problems nowadays [2]. However, little is known beyond the described effects of single stressors on specific ecological endpoints [3] and our understanding of the main causes for the losses of biodiversity still remains vague. Besides the usual taxonomy-based approaches, trait-based approaches are being increasingly used as an alternative to assess stream biological integrity and discriminate the influence of individual antropoghenic stressors and natural influences. Traits may help to reveal the cause of impairment and give an indirect insight into which ecosystem functions may be affected by human disturbance [4]. The objectives of this study were following: a) to assess the risk/presence of multiple stressors in four Iberian rivers and b) to test if we can observe the adverse effects of stressors in the local benthic macroinvertebrate communities c) to determine the difference of taxonomical and functional (traits) diversity changes in relation to stressors present and finally d) to discriminate which stressor or stressors are influencing more than others on the macroinvertebrates. The presence of one or more potential stressors was evident at all the sampling sites. The highest risk was mainly present at the lower parts of the rivers in the area surrounded by agricultural lands or/and urban zones where high concentrations of toxicants, in particularly insecticides, were found. Both organic micropollutants (particularly pesticides) and heavy metals significantly contribute to acute ecotoxicological risk. Besides, most of the sampling sites were characterized by combinations of two or more stressors; such as organic pollution, metals, high nutrient levels and conductivity.

Metals in the Environment: Fate, Speciation and Bioavailability in Water, Soil and Sediment (III)

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A biophysicochemical approach for assessing the dynamics of metal biouptake and toxicity

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Understanding the toxic and essential trace compounds uptake by microorganisms under conditions relevant to natural environment is a major trigger of concern in environmental risk assessments. While toxicity assays are well documented in numerous studies where exposed ambient concentrations of metals are related to toxicology endpoints such as mortality or growth rate, predicting metal toxicity with clear account of the dynamic interplay between cell growth and metal biointerfacial partitioning is still very scarce. We recently proposed an integrative theory where metal transport, adsorption, excretion, internalisation and depletion processes are rigorously accounted for. In addition, the effects of key cell biophysicochemical features (*e.g.* electrostatics or presence/absence of cell surface layer such as EPS) on metal uptake and toxicity were highlighted. In this presentation, the theory is briefly presented together with supporting experimental data collected on bacteria and algae suspensions exposed to Cd(II) solutions. In both situations, it is shown how a critical examination of these data with help of theory can be valuable in deciphering the mechanisms governing the partitioning of metal at biointerfaces over time and accompanied toxicity effects. The theoretical formalism detailed in this presentation clearly highlights the intimate relationships among the metal partitioning dynamics at the cell−solution interphase, the biophysicochemical properties of that biointerphase, and the cell growth inhibition kinetics.

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Toxicokinetic-toxicodynamic model to explain the decreased bioaccumulation but increased toxicity of Cu in an estuarine clam under higher salinities

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Although it is well recogized that salinity affects metal toxicity to estuarine organisms, to quantitatively simulate and predict the effects of salinity is still a challenge. Salinity affects metal toxicity not only through water chemistry, but also by altering the physiology of organisms. The biotic ligand model (BLM), which can only consider the effects of water chemistry, is probabaly not applicable to estuarine waters. Indeed, in this study, we found that higher salinities led to lower Cu bioaccumulation in the euryhaline clam *Potamocorbula laevis*; however, the associated toxicity was not lower but higher. The toxicokinetics of Cu were determined by using Cu-65 as a tracer at 7 salinities, i.e., 5, 8, 10, 15, 20, 25 and 30 psu. Cu toxicity tests were also conducted at these 7 salinities. A two-compartment toxicokinetic-toxicodynamic model was then developed to explain the decoupled effects of salinity on Cu bioaccumulation and toxicity. With the increase of salinity, the uptake rate constant of 65Cu decreased significantly from 0.546 L/g/h to 0.213 L/g/h. The inhibitive effects of salinity on Cu bioaccumulation was consistent with the prediction of the BLM. Elevating salinity from 5 psu to 30 psu significantly raised the killing rate of Cu from 0.60 mg/μg/h to 2.08 mg/μg/h, while did not significantly affect the internal toxic threshold of Cu. This indicated that the excessively accumulated Cu manifested higher toxic effects to the clams at higher salinities, probably due to their higher osmoregulatory stress. Using the toxicokinetic-toxicodynamic model, we can quantitatively predict the effects of salinity on both Cu bioaccumulation and toxicity. This model can serve as a useful tool for assessing ecological risks of Cu in estuary waters. **Key words:** salinity, copper, toxicokinetic-toxicodynamic model

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Behavior and toxicity of silver nanoparticles and silver nitrate in aquatic indoor microcosms

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In this study effects of AgNPs and $AgNO_3$ are investigated to assess toxic effects of AgNPs and Ag^+. Therefore the release of Ag^+ from AgNPs and $AgNO_3$ and the accessibility of Ag^+ in the water phase is researched in separated microcosms with a volume of 1 m^3. The microcosms contain natural sediment (0.25 m^3), surface water (0.75 m^3), zooplankton and phytoplankton. AgNPs with a mean diameter of 20 nm and $AgNO_3$ were applied into aquatic indoor microcosms at four different target concentrations, respectively. In addition four control microcosms were used. During a period of 100 days target concentrations were maintained by regular test item additions. The recovery was followed over additional 150 days. To allow observation of toxic effects, data of cell density were collected. The aim of this near-to-nature approach is to give an as precise as possible picture of nanoparticle behavior and toxicity on aquatic communities and to enable a direct comparison to effects caused by $AgNO_3$. 78 days after start of the exposure, maximum concentrations of Ag^+ were observed for both AgNPs and $AgNO_3$test systems. Independently from the initial AgNP loading no higher concentration of Ag^+ could be measured in the water phase. At the current point of the study evaluation results of cell density measurements indicate a phytoplankton growth in AgNP treated microcosms and a potential degradation of zooplankton. The exposure to 5 μg/L AgNPs lead to an exponential growth of phytoplankton until day 42. This effect seemed to be induced by a degradation of zooplankton. The application of lower AgNP concentrations (0.078, 0.313 and 1.25 μg/L) did not lead to changes of the amount of cells in the water phase. This may indicate that the zooplankton is not directly affected by exposure to AgNPs. Nevertheless, after stopping the test item additions similar amounts of cells could be found in the water phase of all treated microcosms and controls. Apparently similar communities were present in all microcosms and the recovery of the communities occurred in spite of the presence of remaining silver in the system.

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Arsenic induces changes in gut microbiota associated to mucus in vitro models

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Arsenic (As) is an environmental contaminant widely distributed through the Earth crust. The main route of exposure to inorganic arsenic, the most toxic arsenical specie in contaminated water and food, is via oral. Arsenic absorption occurs mainly in the small intestine, however non-absorbed arsenic can reach distal gastrointestinal regions. Human trials showed that 44-60% of arsenic ingested after rice consumption was excreted through urine in five days, so the remaining amount of As could reach colon or be retained in the human body [1]. Therefore the colonic absorption of arsenic can contribute to As internal exposure and, moreover, arsenic could interact with distal gut microbial community. Gut microbiome, therefore, should be considered as an environmental factor affected and, also, influencing arsenic toxicity. This research evaluated in vitro the effect of environmentally relevant doses in mucus associated gut microbiome using batch assays and the simulator of the human intestinal microbial ecosystem (SHIME), including the mucus environment (mucus-SHIME, M-SHIME). Faecal samples from healthy individuals were inoculated in batch assays and M-SHIME reactor and exposed to arsenic (10 and 100 μg/l). After different time points, the metabolic activity of the gut microbiota was assessed by quantifying short chain fatty acids, branched short chain fatty acids and ammonium contents. Structural changes on mucosal microbial community was performed by polymerase chain reaction (PCR)-denaturing gradient gel electrophoresis (DGGE) of the 16S ribosomal RNA (rRNA) gene and quantification of 16S rRNA gene copy numbers by real-time PCR (qPCR). The results obtained show that arsenic exposure affected significantly the branched short chain fatty production in fed batch and M-SHIME experiments, indicating changes in the metabolic activity of gut microbiome related with the protein metabolism.The DGGE fingerprint of total bacteria in alginate mucus beads was significantly affected after arsenic exposure after processing gel bands with Bionumerics software. The results presented in this work suggest that the gut microbiome is an environmental factor to consider in risk assessment of arsenic exposure. The health consequences of arsenic interaction with gut microbiota need further research. The mucus associated bacteria are suggested as a key element in the arsenic – host interplay.

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Copper-induced changes in intracellular thiols in marine algae

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Toxicity of metals to aquatic organisms is dependent on both external factors, such as exposure concentration and water quality parameters, and intracellular processes including specific metal-binding sites and detoxification. Current models used to predict copper (Cu) toxicity in microalgae do not effectively consider the intracellular processes. This research examined the toxicity of Cu towards microalgae by investigating the intracellular Cu-binding ligands of phytochelatins and glutathione from four species of marine microalgae exposed to Cu at their respective IC_{50} (concentration of Cu required to inhibit population growth by 50 %) at intervals over 72-h. IC_{50} values were chosen to represent equal amounts of cellular stress across the four species despite their differences in Cu tolerance. Despite similar Cu tolerances in *Phaeodactylum tricornutum* and *Ceratoneis closterium*, differences in internalised Cu, phytochelatin production and reduced glutathione were observed. *P. tricornutum* maintained reduced glutathione at 58 - 80% of total glutathione levels throughout, whereas in *C. closterium* reduced glutathione constituted < 10% of total glutathione after 48 h of Cu exposure. *P. tricornutum* internalising significantly less Cu but produced more phytochelatins and of longer chain length than *C. closterium*. Two green algae, *Dunaliella tertiolecta* and *Tetraselmis* sp. had very different Cu tolerances, and also exhibited differences in internalised Cu, phytochelatin production and reduced glutathione concentrations. *Tetraselmis* sp. internalised three times more Cu than *D. tertiolecta* and had significantly more intracellular thiols. In both green species a decrease in the reduced:oxidised glutathione ratio was observed following Cu exposure. Phytochelatin production was markedly different between acute (< 24 h) and chronic exposures (24 – 72 h), with shorter chain lengths dominating for chronic exposures. Reduced glutathione increased slightly at longer exposures in both species, potentially indicating a decrease in intracellular oxidative stress despite a consistent increase in intracellular Cu. This work has shown that the intracellular reaction to Cu is species specific, and responses were not necessarily related to their respective tolerances, or the amount of intracellular Cu. This suggests that there may be multiple modes of Cu toxicity and that detoxification processes vary between species.

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Time-course metabolomics responses of transplanted oysters Crassostrea hongkongensis to estuarine metal pollution

C. CAO, Hong Kong University Sci. Technol.; W. Wang, Hong Kong University Sci. Technol. / DIVISION OF LIFE SCIENCE

In a dynamic and fluctuated estuarine environment, heavy metal toxicity is greatly influenced by various environmental stressors, increasing complexity and difficulty of accurately predicting potential biological/ecotoxicological effects simply based on indoor laboratory experiments. In this research, metabolomics responses of oysters *Crassostrea hongkongensis* were characterized under metal contamination in an estuary. Oysters were transplanted from a clean site to multiple-metal-contaminated sites in an estuary for six months. Both metal concentration and metabolomics responses in oysters' digestive glands were investigated over different periods of exposure (0 week, two weeks, four weeks, and six months). Following transplantation, all metals (Ni, Cr, Pb, Cd, Cu and Zn) in digestive gland tissues showed an elevated trend over time. Cu, Zn and Cd were dominant polluted metals. Using ^{1}H NMR metabolite approach, we demonstrated that significantly varied metabolites were mainly responsible for osmotic regulation, energy metabolism, nucleotides and glycerophospholipid metabolism. Within initial four weeks of exposure, the amount of amino acids and osmolytes was increased for osmotic regulation. Addtionally, energy demand was decreased to accumulate glucose and glycogen for adaptation. However, oysters mobilized early accumulated amino acids and energy storage compounds to produce ATP and maintain a normal physiological function over a longer period of exposure. The metabolomics study demonstrates that oysters adopt different strategies to maintain living status in a heavily-metal-contaminated environment.

Modelling of pesticides and biocides fate and exposure in a regulatory context

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Impact of updated modelling methodologies on pesticide E-fate risk assessments

A. Boivin, ANSES

Regulation 1107/2009 states that active substances used in plant protection products are only approved in the EU if it may be expected that their use will not have any harmful effects on human and animal health or the environment. In practice, risk assessment is performed for the active substance (at European level) and for the product containing the active substance by EU member states (in the context of the Zonal procedure). While regulation may provide indications on the data requirement and the protection goals, procedure on how to derive predicted environmental concentrations in the different compartments is made in guidance documents publicly available. Such guidances are valuable in order to improve the risk assessment methodologies. It's also noticeable that guidances are also updated or complete with aim to achieve a high scientific quality in the risk assessment. In addition they may help to harmonize the risk assessment carried out for pesticides at EU and zonal level. In the environmental fate and behaviour area, at least 9 guidances are available covering the various compartments, process and crop type. Anses proposes some feedbacks of guidance implementation and the practical or foreseen consequences on risk assessment. It includes worked examples to identify sensitive topics highlighted during risk assessment performed by member states. Consequences for the environmental fate and behaviour (and ecotoxicology) sections and the associated risk assessment are discussed. Benefits and drawbacks are discussed.

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Can the Danish regulatory modelling approach assess the leaching risk of pesticides and their metabolites as monitored via the Danish Pesticide Leaching Assessment Programme?

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The regulation of pesticide use in Denmark is based on the European Union (EU) registration processand guidance documents for the Northern Zone and Denmark. As described in these guidance documents, the Danish assessment of the risk of leaching of pesticides to groundwater is based on modelling with the FOCUS Hamburg model scenario (PELMO) or the two national model scenarios Karup and Langvad (MACRO). The approach taken by Denmark also differs from that of the EU with respect to consideration of relevancy of metabolites, selection of degradation rate and sorption parameters and the evaluation of predicted environmental concentrations in groundwater. In Denmark, if modelling indicates leaching below, but close to the threshold of 0.1 $\mu g\ L^{-1}$, the compound's leachability may be evaluated in the Pesticide Leaching Assessment Program (PLAP). PLAP is an intensive monitoring programme evaluating the risk of leaching of pesticides and/or their metabolites under field conditions. PLAP today encompasses five fields representing various types of soils and near surface geology where the pesticides are used at a realistic worst case dose and according to good agricultural practice. The PLAP-fields have been thoroughly characterized (e.g. pedology, hydrogeology) and monitored as regard to the water balance. Modelling was carried out according to the Danish and EU approach for 27 pesticides and 19 metabolites that have been tested in PLAP. The modelling results were compared to the findings in the groundwater wells in PLAP. As expected there are differences between the Danish and the standard EU approach with regard to the modelling results. Due to the more conservative input and more conservative approach for interpreting output, the results for the Danish approach generally predicts a greater risk of leaching. Comparison with the results for PLAP reveal that in some cases the models over-estimate the leaching risk. The potential causes for this discrepancy between simulated and observed leaching scenarios will be explored. **\n**

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Drainage models and macroporous soils

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In the procedure for the registration of plant protection products at the EU level and at the member state level, simulation models are used to assess the fate of these products in the soil. Preferential flow is an important process which can significantly affect the leaching of plant protection products through soil and discharge via drains into surface waters. A scientific SETAC Workshop was organised in Vienna, Austria, from 23 to 24 October 2014 on drainage models and macroporous soils. Scientists that are actively involved in research on the fate of plant protection products in macroporous soils were invited to participate. In addition, representatives from industry, registration authorities and consultancies were also invited. The focus of the workshop was on the validation status of models that are available at present for the description of the fate of plant protection products in macroporous soils and to make recommendations for improvement of model concepts for macropore flow in soils as well as to elaborate the data requirements needed to test the improved model concepts. The models that were considered in detail were HYDRUS, MACRO and SWAP/PEARL. In key note presentations the state of the art in this research domain was presented. The workshop consisted of plenary sessions, in which participants were given the opportunity to give a short presentation on the topics to be addressed in the session and break-out sessions to discuss specific subtopics. The differences in conceptual approaches, options to consider dual permeability and dual porosity systems, equilibrium and non-equilibrium domains and options for bottom boundary conditions in the HYDRUS, MACRO and SWAP/PEARL models were discussed. Important fields that need further attention are the contribution of biopores to preferential flow. Further, the current description of surface processes in the models requires further attention, including the temporal variation of soil properties, which govern the infiltration of water. Tillage and traffic effects on macroporosity are currently not considered in the modelling, so concepts need to be developed that describe these processes in a realistic way. Further, more efforts are needed to test the improved models and as well as on elaborating the type of measurements needed to test these models in the field.

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Options to consider during accumulation calculations for predicted environmental concentrations in sediment
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Predicted environmental concentration in sediment (PECsed) for regulatory purposes is currently calculated according to the FOCUS surface water report. The standard time frame of a FOCUS surface water simulation (12-16 months) could potentially limit the estimation of chemical accumulation in sediment. TOXSWA, however, is not currently parameterised to run over longer periods within current the regulatory framework. In this work we aim to identify the main processes which drive accumulation in the sediment and to discuss options to consider during accumulation calculations for regulatory exposure assessments. We considered a slowly degrading sorbative compound applied annually to winter wheat. The FOCUS surface water run-off scenerios were used to calculate the PECsed for one year and for 20 years. A second analysis was performed to examine sediment burial within run-off scenarios for annual application to winter wheat.Soil mass input and sediment chemical mass over 20 years were generated using SWASH. A simple, conservative assumption was made that 100% of the soil adsorbed chemical and erroded soil mass is deposited to the sediment along with partitioning to and from the water column. Applying the stratigraphic principle of superposition a stratigraphic column spanning 20 years was constructed. Both the stream and pond scenarios demonstrate that dissipation in the sediment compartment is dominated by desorption/back-diffusion. Neglecting dissipation by desorption and back-diffusion leads to overestimation of PECsed by a factor of 2.5 to 4. In the second analysis the constructed stratigraphic column demonstrates that in some cases >20cm of sediment would be deposited during a 20 year simulation. Furthermore, chemical mass would be distributed heterogenously throughout this deposited thickness and not solely within an upper 5 cm or 1 cm compartment. Neglecting the processes decribed here leads to overestimation of the potential accumulation in the sediment. Desorption and back-diffusion as a major sediment dissipation process is already considered in the current exposure assessment scheme over a 12-16 month time period. It could and should be used to quantify accumulation over longer time periods more realistically. Additionally, when calculating PECsed over a longer period the option of simulating burial of sediment should be available as a more realistic, higher-tier option for calculating sediment PECs within a regulatory context.

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Effect of long-term sorption kinetics of pesticides on their simulated leaching to groundwater
J. Boesten, Alterra / ERA team
Assessment of leaching to groundwater is an important aspect of the risk assessment of pesticides within the EU. The lower tiers of this assessment are based on calculations with simulation models. The first tier is commonly based on the assumption of equilibrium sorption based on batch adsorption studies with a typical equilibration time of 24 h. In the past decade, interest has increased to include long-term sorption kinetics (LTSK) as a higher tier. It is therefore interesting to assess the likely magnitude of the decrease in leaching concentrations due to inclusion of LTSK. Calculations were made with FOCUS_PEARLv4.4.4 for the FOCUS Okehampton groundwater scenario with an annual application of 1 kg/ha in winter wheat one day before emergence. This was done for a range of substances using regular grid of K_{OM} and *DegT50* values; the K_{OM} ranged from 15 to 210 L/kg and the *DegT50* from 5 to 70 d. The desorption rate coefficient k_d was 0.025 d^{-1} and the ratio of the non-equilibrium Freundlich coefficient divided by the equilibrium Freundlich coefficient (f_{NE}) was 0.5. The Freundlich exponent N was set at either 0.7 or 0.9. There is a complication with respect to the parameterisation of the *DegT50* because the overall degradation rate is a function of $k_d$and f_{NE}. So if the effect of $k_d$and f_{NE} on leaching is studied while keeping the same *DegT50*, this would lead to comparing leaching of substances with different overall degradation rates which is not meaningful. This problem was overcome by simulating for each substance the decline of the mass of substance in a hypothetical laboratory degradation rate study with the Okehampton top soil at field capacity and 20°C and by deriving the first-order overall degradation half-life of this substance from a fit to these simulated data. Both for $N = 0.7$ and $N = 0.9$, for the vast majority of the *DegT50*-K_{OM} combinations the effect of including LTSK was closely related to the level of the leaching concentration: the lower the leaching concentration of the substance, the higher the effect of including LTSK. The effect of including LTSK was found to be considerably larger for $N = 0.7$ than for $N = 0.9$: for $N = 0.9$, a leaching concentration of 0.1 μg/L with LTSK corresponded with a concentration of about 1 μg/L without LTSK whereas for $N = 0.7$, 0.1 μg/L with LTSK corresponded with 3-8 μg/L without LTSK. \n

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The EFSA PPR Statement on the FERA Proposal for Aged Sorption Guidance
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Aged sorption (also referred to as non-equilibrium sorption) refers to the increase of sorption of a substance over time. Although aged sorption is a generally accepted scientific phenomenon, the procedures to derive parameters of the aged sorption process are uncertain. The interest in aged sorption led to the inclusion of recommendations relating to the use of aged sorption parameters in the revised FOCUS groundwater report. However, the extent of investigation into this subject was limited and a general recommendation was made that default parameters for aged sorption could be adopted at higher risk assessment tiers for any substance. Separate experiences with the difficulties in considering aged sorption data in regulatory risk assessment triggered the UK Chemicals Regulation Directorate (CRD) to charge FERA (the Food and Environment Research Agency) with the development of guidance on study conduct and generation of aged sorption parameters suitable for use in regulatory risk assessment. The scientific Panel on Plant Protection Products and their residues (PPR Panel) was asked to prepare a scientific opinion on the FERA guidance proposal. The experimental and modelling approaches in the proposed guidance are reasonable compromises between the required effort and what is desirable from a theoretical point of view. The Panel has, however, concerns about the interpretation of the experiments and how results of the experiments should be used in the leaching assessment. The Panel investigated options for improvement. Evaluation will be finalised after the guidance document has been revisited by the authors..

Prospective and retrospective soil risk assessment of chemical stressors

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Utility of a site specific soil microbial health assessment test suite to assess the impact of single chemicals on soil microorganisms for risk assessment
L. Beaudette, Environment Canada / Ecotoxicology and Wildlife Health Division; A.D. Samarajeewa, A. Crossman, Environment Canada; S. Kvas, Environment Canada / Biological Methods; R. Scroggins, Environment Canada
Soil microorganisms are an integral part of the terrestrial ecosystem playing a significant role in nutrient cycling and organic matter decomposition. As such, impairment of the soil microbial function and diversity, due to a contamination event, can lead to impacts at higher trophic levels over a period of time. To assess the impacts of soil contaminants on soil microbial function and diversity, Environment Canada (EC) developed a site specific soil microbial test suite. The test suite evaluates soil microbial biomass, activity, and community structure using the following tests: heterotrophic plate count, fumigation-extraction, nitrification, organic matter decomposition, substrate-induced respiration, basal respiration, bait lamina, enzyme assays, community level physiological profiling, and next generation DNA sequencing. Initially, the test suite was used to determine the impacts of site specific boreal forest contamination events (polycyclic aromatic hydrocarbon, metals) on the soil microbial community; however, until recently, the utility of the test suite has been realized for laboratory risk assessment research. Data will be presented on the use of the microbial test suite on healthy sandy loam reference soil spiked with different concentrations of chemicals. In contrast to the site specific results where EC50 values are not possible to calculate, risk assessment EC50 results will be presented to inform risk assessors of the impact of the chemicals on the soil microbial community.

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Topical Scientific Workshop on Soil Risk Assessment (Helsinki, October 2015); problem definition and conceptual model
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Multidisciplinary and international collaboration is essential for developing a science-based methodology for risk assessment of chemicals released to or reaching soil. Topical Scientific Workshop on Soil Risk Assessment brought over 200 experts representing industry, academia, regulators and other stakeholders from all over Europe and across the world in order to discuss the state of the art in assessing the impact of industrial chemicals, biocides and plant protection products in soil. The overall aim was to identify the most critical improvement needs for soil risk assessment, and to identify in which cases the harmonisation of different regulatory approaches would bring an added value. Three main topics were discussed during the two-day workshop: Problem definition and conceptual model for soil risk assessment, Environmental exposure and fate assessment, and Effect assessment. Several areas where identified were harmonisation of the approached in scientific assessments for pesticides, biocides and industrial chemicals under REACH and international collaboration would bring added value for the soil risk assessment. This presentation will focus on the conclusions reached in discussion on the problem definition and conceptual model for soil risk

assessment and describe identified developments needs within regulatory framework. Following conclusions and development needs will be elaborated; It was seen as beneficial if technical development of specific protection goals (SPG's) could be harmonised across the industries and regions, if and where applicable, with clear definition of land/soil use, product use, exposure scenario and time scale. Ecosystem services approach was considered as useful communication tool in defining what do we want to protect. Considering bioavailability in soil risk assessment and defining concept of biodiversity (including functional biodiversity) in regulatory context. Combining exposure assessment – how the chemicals enter into and behave in the soil – with effect assessment – how the chemicals then affect different organisms. Additionally, other identified regulatory science-oriented R&D needs such as the application of the equilibrium partitioning method, a screening level for soil risk assessment, using information from aquatic organisms, and a calibration based on information on higher-tier studies will be discussed in more detail.

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Translation of Ecosystem Services based Protection Goals into an appropriately calibrated tiered Collembola risk assessment of plant protection products - case study: lindane

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Soils provide relevant Ecosystem Services (EsS) e.g. by regulating water and nutrient cycles which have a direct impact on the EsS food production. In agricultural landscapes the biodiversity and the fertility of soils have to be protected from an unacceptable impact from PPP. However, landscapes are heterogeneous and different ecosystem services are provided by different parts of the landscape. Thus, a spatial differentiation needs to be taken into account when setting protection goals. Due to conflicting needs of different EsS it is not possible to protect biodiversity in all areas and all the time. Therefore, biodiversity should be protected on landscape level. Thus, the risk assessment should differentiate between 1) an off-field risk assessment where the focus is on protection of biodiversity and 2) an in-field risk assessment which should focus on protection of those soil functions which are directly related to the most relevant EsS for agricultural areas (i.e. food production). In order to improve the current toolbox for soil functional test systems that could be used in an in-field soil risk assessment a project was initiated by the European Crop Protection Association in 2014. Different functional test systems (litterbag test, minicontainer test, bait lamina test) are tested on their sensitivity to different insecticides in a field trial and evaluated with regard to their relevance for the EsS provided by soils, their degree and potential for standardization, and their suitability for being used in the risk assessment. The case study is focusing on one specific group of soil organisms (Collembola) and the ecosystem services they provide by examining different structural and functional tests in a tiered risk assessment approach. Laboratory tier 1 test will be compared with an intermediate tiered two-generation test, and higher tier field effect data on both a structural and functional level. We want to discuss how protection goals derived from the Ecosystem Services approach translate into an appropriately calibrated tiered risk assessment approach for collembola.

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Topical Scientific Workshop on Soil Risk Assessment (Helsinki, October 2015): environmental fate and exposure assessment

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The main principles and elements of exposure assessment of chemicals in the soil are known and used accross various legislations. However some of the properties and processes should gather more consideration in the exposure assessment than is currently the case. Specific attention should be given to the substances possessing specific structures (e.g. structures that earmark a substance as PBT) or physico-chemical properties. The lack of realism in the fate testing requested under current regulatory frameworks, leads to the difficulties when the testing data are employed for the simulation of processes under real environmental conditions. In the workshop it was advised that the link between fate and exposure assessment on the one hand and hazard assessment on the other hand should be further improved. It was concluded that the bioavailability of the chemicals should be further understood and implemented in the regulatory framework. The possible harmonisation of the approaches and models used today for the soil exposure assessment was discussed by the participants of the workshop. The use of modelling tools for the exposure assessment and risk assessment in general has a great potential for further development and practical use, but the limitations in the use of available/newly developed tools needs to be well understood. The sharing of experiences and harmonisation of approaches for the fate and exposure assessments within different legislations was seen as a key possiblity for further improvement of the relevance and reliability of the soil risk assessments. Discussions on NER formation and bioavailability resulted in the conclusion that the topic has potential for the implementation into regulatory exposure assessment schemes. However, some concerns around the topic were raised. It was concluded that higher realism of the fate/exposure assessment (regardless whether it is based on laboratory/field measured or modelled data) and its linking with results of hazard assessment is necessary. There were specific proposals for initiating activities for the development of more realistic approaches/methods for soil exposure assessment taking into account additional parameters (physico-chemical) of substances and of the medium (soil) as well as integrating into the assessment methodologies other processes (e.g. desorption) and conditions relevant for the real environment. Research needs and the directions for development of the regulatory science were identified.

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Validation of a New Environment Canada Test Method for Measuring Contaminant Effects on Survival and Reproduction of Soil Mites using Oppia nitens

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In 1995, Environment Canada (EC) initiated a multi-year program to develop biological test methods that could be used to assess the toxicity of contaminants in soils using terrestrial organisms. The goal was to develop test methods that were applicable to Canadian soil types using species that were representative of Canadian soil ecosystems. The creation of a new Environment Canada test method for measuring survival and reproduction effects in contaminated soil using an oribatid mite is the latest soil testing methodlogy to be developed into a national standardized method. The principal procedure is a definitive 28-day test measuring chronic toxicity using adult survival and number of live juveniles as endpoints. The mite species recommended as the test organism of choise is *Oppia nitens* (C.L. Koch, 1839), a species found in surface soils across Canada. This toxicity test is based on EC research conducted by staff of the Soil Toxicology Laboratory. An inter-laboratory validation study will be initiated in January 2016 as part of the EC method standardization process. Eleven ecotoxicology laboratories from across Canada and Europe have volunteered to participate in this method ring testing effort. Participating laboratories have been establishing their *O. nitens* cultures for the past three months following standard operating procedures developed by EC. Three rounds of testing are planned for this method validation project which is schedule to begin in late January 2016. As a parallel effort, Environment Canada has begun formal method writing with the goal of having a 1st draft of the official test method completed before completion of the ring testing program. An overview of the test method and early results of the inter-laboratory testing program will be presented.

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Outcome of the Topical Scientific Workshop on Soil Risk Assessment (Helsinki, October 2015): state-of-the-art and new developments in soil effect testing

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The primary objective of this workshop was to review the state of the art regarding soil risk assessment in view of developing updated scientifically-sound principles and approaches for assessing ecological risks of chemical substances, which are released to or reach the soil. The workshop also provided a platform for academia, regulators and other stakeholders to address how the main long-term challenges from the regulatory perspective can be reflected and employed in the current and future research topics on soil risk assessment. The discussions were reinforced by information on the recent scientific developments and on risk assessment methodologies applied in chemicals management both within and outside the European Union. There was a common understanding that the Ecosystem Services (ES) approach can be a suitable conceptual framework to define specific protection goals in order to ensure the protection of the environment including biodiversity. This process encompasses the identification of ES provided by the soil system and associated species or communities responsible for the provision of these services (SPUs) through a dialogue with risk managers. Afterwards, a process for setting reference tiers (allowing direct comparison with the protection goals) and to calibrate down for intermediate and lower tiers, is needed. Particularly for the lower tiers, it has to be checked whether standardized test methods with those relevant species (SPUs) are available. If not, alternative test species and test methods have to be identified. Very few standard higher-tier tests are available. Finally, a list of recommendations was prepared by the workshop participants, providing ideas on how to proceed regarding soil effect test methodology in the light of the new risk assessment approach. During the workshop already several topics were identified where harmonisation of the approaches and international collaboration would bring added value for the soil risk assessment such as, just to mention a few, defining specific protection goals,

considering bioavailability in soil risk assessment and defining the concept of soil biodiversity in the regulatory context. In addition, already concrete proposals were made about launching activities on defining boundaries for applicability of the Equilibrium Partition Method approach, considering non-extractable residues, application of Species Sensitivity Distributions (SSDs) and revisiting assessment factors (AFs).

Life Cycle Data and Modeling Developments - From Data Collection to Usage (III)

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A model and tool to calculate life cycle inventories of chemicals discharged down the drain
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A new model and tool to calculate life cycle inventories (LCIs) of chemicals discharged down the drain is presented. Its main innovation is the fact that it attributes the exchanges with the technosphere and the environment taking into account the expected behaviour of individual chemicals in the wastewater treatment plant (WWTP) and the environment, thus contributing to better decision making and data availability in the context of the life cycle of chemicals. The model covers treatment of organic and inorganic chemicals and input variables by the user include chemical-specific data (composition, phys-chem properties, half-lives, and fate factors in the WWTP) as well as scenario variables (% population connected to WWTP, country, nutrient removal in the WWTP, sludge disposal scenario including landfill, incineration and agriculture). The WWTP is modelled with a mass and energy balance taking into account the partitioning of the chemical to air, sludge, treated effluent, and depending on its degradability, the transformation by microorganisms to CO_2 and new biomass (excess sludge). Sludge is treated by means of anaerobic digestion and the fate of any fraction of chemical released to the environment (e.g. treated effluent, direct discharges) is assessed with a previously developed model, which calculates greenhouse-gas (GHG) emissions following degradation in environmental compartments. Besides GHG emissions, emissions of nutrients (N, P) are also considered. Concerning sludge disposal by incineration and landfilling, this is assessed by means of the models developed for the ecoinvent database, whereas reuse of sludge in agriculture is accounted for by means of tier 1 emission factors from the IPCC. The model is programmed in an Excel spreadsheet, the WW LCI tool, which accommodates simultaneous calculations for 30 chemicals, either individually or as a mixture. The output of the WW LCI tool is a comprehensive LCI linked to ecoinvent v3 data sets, that can be imported to LCA software in order to complement a life cycle assessment study of a particular chemical or a chemical mixture associated to a product or service. This model is the first one to address a chemical-specific and comprehensive LCI of chemicals discharged down the drain, including all the described processes. It constitutes an advance over previous models using generic descriptors like BOD, COD, etc. and constitutes an advance in how the end-of-life stage of chemicals is addressed in LCA.

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Typology of mixes for world electricity production inventory for CLCA
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Over the past two decades, consequential LCA (CLCA) has emerged as a modelling approach to assess consequences of either direct or indirect changes. The development of CLCA has received a lot of interest from practitioners and requires specific inventory data. These data, often based on economic models are long to set and require a good understanding of processes. Moreover, no robust generalised technical databases for consequential LCI about indirect changes have been provided yet, especially in the case of electricity production, which may lead to uncertain LCIA results. In that context, the development of more generalized LCI data represents a major challenge for consequential LCA application. A first step to address this issue is to better understand electricity production mix worldwide. So, the aim of this work is to set a typology of electricity production mixes. The proposed typology is based on the analysis of two factors: the former involves the composition of electricity production mixes of 94 countries producing more than 10 TWh in 2012[1] and the latter is their calculated GHG (gCO_2eq/kWh) assessment from LCA using IPCC 2013 LCIA method[2]. The analysis of these two characteristics leads to the determination of a 4-group typology: very low (0-37), low (37-300), mean (300-600) and high (>600) emitting countries, each sharing general characteristics about electricity power generation, i.e., hydraulic/nuclear, hydraulic/nuclear and gas, gas, and coal/ fuel. The next step of our work is to study the dynamic evolution of some countries selected from each group of the proposed typology, using historical data. The first case study will be the France energy system. The comparison of historical and contemporary observed production data, as previously carried out in the literature[3] will help us to identify the uncertainty remaining in evaluating electricity production processes, and so the consequences of integrating such data in consequential LCI. \n[1]The Shift Project. 2012. TSP Data Portal. tsp-data-portal.org. Accessed 03/16/2015. [2]IPCC, 2013: Climate Change 2013: The Physical Science Basis. Cambridge University Press, Cambridge, United Kingdom and New York, 1535 pp [3]Mathiesen et al., « Uncertainties related to the identification of the marginal energy technology in consequential life cycle assessments », J. Clean. Prod., vol. 17, n°15, p. 1331-1338, oct. 2009

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Multiple recycling loops in attributional LCA - Misconceptions and solutions
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Modelling of recycling in LCA has always been an important topic for discussion. It is often argued that the modelling method should reflect the benefits of multiple recycling loops. Some guidance on this area is given in ISO/TR 14049: the avoided burden method is proposed if the material is recycled without loss of inherent properties, and the number of (future) uses of the material is used to calculate the inventory per life cycle when the material undergoes changes in its inherent properties. However, it is questionable whether this guidance is suitable for attributional LCA. In the attributional database of ecoinvent 3, the life cycle inventory is partitioned over the co-products of multifunctional processes by "allocation at the point of substitution". We show that this method is equally applicable to materials that are recycled at the end of life. We propose a new formula that calculates the LCI of one life cycle of a material, considering the consumption and production of co-products and recycled materials in that life cycle. If the consumed recycled material has been recycled several times before, the formula can be solved to calculate the inventory after a definite or infinite number of recycling loops. The adapted formula can be applied when the material is recycled via a material pool where the inventory regarding the primary production, recycling activities and waste treatment are similar in other product systems, and the average recycled content and the end-of-life recycling rate in the material market can be determined. The LCI after a large number (>5) of recycling loops corresponds to the avoided burden approach as described by ISO/TR 14049, only when the recycled content and the end-of-life recycling rate are equal. This is not a realistic condition for most materials. Therefore, it is important to distinguish these rates, which is not done in the current application of the avoided burden method. Other methods that consider multiple recycling loops often refer to downstream recycling activities. We show that only multiple recycling loops in the past are relevant in an attributional LCA. Whether the produced recycled material at the end of life can be recycled again in the future, is outside the scope of an attributional LCA that focuses on one product life cycle.

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Methodological Guidelines for the Life Cycle Inventory of Agricultural Products
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In 2012, the sustainability consultancy Quantis[1] and Agroscope[2], the Swiss Federal research institution for agriculture, joined their forces to create a unique international consortium of nine major, global, private companies from the agro-food value-chain[3] and two governmental agencies[4] into what would become the World Food LCA Database (WFLDB) project. Three years later, 400 datasets for crops, animal products and food products in 40 countries were delivered to the project partners and submitted to the ecoinvent Centre for publication in 2016 and 2017. Thereby, the entirety of the project developments are made available to a broad public of LCA and sustainability practitioners. A major outcome of WFLDB was the development of the *Methodological Guidelines for the Life Cycle Inventory of Agricultural Products**[5]***, a detailed and comprehensive guidance for inventory modelling of agricultural and food systems. To date, these are among the very few – if not the only – peer reviewed documents providing detailed and operational guidance applying to a wide variety of agricultural systems and countries, from farm to plate. The *Guidelines* are based on existing scientific emission models and are compliant with the most recognised standards (ISO, ILCD, ecoinvent, IPCC). They are also to a large extent aligned with draft Product Environmental Footprint Category Rules (PEFCR) for several sectors. All key methodological issues relevant to agro-food systems are addressed, such as the definition of system boundaries, co-products allocation, inventory modelling for fertiliser and pesticides, emission flows, water modelling and land use change. A unique set of data collection principles combining statistical data from FAO and agronomic extrapolations was applied to generate consistent LCI data. Innovative approaches were developed where no global consensus is yet available, such as the modelling of land use change. The *Guidelines* were first made public in August 2014 and then updated in July 2015. The current version (v3.0) aims to serve as an open reference for LCA practitioners and LCI database developers. The second phase of the WFLDB project, starting in 2016, will further work on improving datasets and expanding these guidelines in cooperation with all stakeholders. Thereby we consider the latest methodological developments and global consensus on key topics such as pesticides emissions modelling, land use change or carbon sequestration in

grassland.

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Process simulation to improve LCI modelling of new technologies - Case study on source-separated urine treatment
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Data collection for Life Cycle Assessment (LCA) of new technologies appears to be challenging (instability of lab/pilot scale, up-scaling uncertainties). The integration of these computational tools within Life Cycle Inventory (LCI) models could be beneficial to the stability and consistency of LCA. This line of research has been followed within the FP7 project ValuefromUrine, where an innovative urine treatment (VFU) has been developed to recover nutrients and increase Waste Water Treatment Plant (WWTP) efficiency. Three scenarios are tested: (i) *Reference* (conventional WWTP), (ii) *small VFU* (pilot scale VFU installed in buildings treat 10% of urine) and (iii) *large VFU* (up-scaled VFU integrated in WWTP treat 50% of urine). The functional unit is the treatment of 1 m^3 of wastewater. Foreground processes are simulated through Python functions for VFU technology (mass balances, electrochemistry, engineering) and SUMO process engineering tool for WWTP. These models, currently under validation, depend on input variables (design parameters, scenario variables, assumptions) for which uncertainty can be defined. The LCI outputs are integrated in the open-source Brighway2 tool. The fertilizers and energy recovered are assumed to avoid the production of conventional market products. Preliminary results, focusing on VFU technology, shows the influence of molar ratio dosing Mg:P on LCI data (other design parameters will be later analysed). Increasing this ratio generates additional impacts linked to magnesium chloride consumption but is also beneficial regarding the avoided production of phosphate-based fertilizer. The environmental profile of VFU technology is mainly driven by the consumption and transport of chemicals, polypropylene (in infrastructures) and the avoided production of fertilizers. The main innovation in this work is the integration of process simulation tools with LCI modelling, in order to cope with the lack of data for new technologies (e.g. use of complex and reliable modelling tools) and to improve results interpretation (data relationships, uncertainty analysis, etc.). Furthermore, this approach, coupled to an optimization algorithm, could determine the optimal design parameters. The authors also stress the difficulty to perform advanced modelling and analyses with commercial LCA software. The use of an open-source platform such as Brightway2 solves the problem but requires programming competences (potential barrier for LCA practitionners).

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Impacts of severe wet-weather events: How to account for temporal variability of unmanaged peak flows in an urban sewage treatment system?
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In typical life cycle impact assessment (LCIA) studies of urban wastewater systems (UWS), average conditions are modelled but there are many annual flooding events with releases of untreated sewage. Such peak conditions are not considered and present a high temporal variability which is not currently accounted for. In addition, the aggregation of the loads from several storm events could bring an issue for the impact assessment on the aquatic categories of eutrophication and ecotoxicity. Hence we are investigating the contributions of these wet weather-induced discharges along with the inclusion of temporal variability in the life cycle inventory (LCI) for UWS. In the framework of the OPUR research programme (Observatory of Urban Pollutants) and in collaboration with the Paris public sanitation service (SIAAP), this work aimed at identifying and describing contributing flows from the UWS in the Paris area by a selection of routine wastewater parameters and priority pollutants. This collected data is organized according to archetypical weather days over a 24-hr span. Secondly, for each archetypical weather days and its associated flows to the receiving river water (Seine) the parameters of pollutant loads (statistical distribution of concentrations and volumes) are determined with statistical treatment of data. Then, the inventory flows (i.e. the potential loads from the UWS) can be used as inputs in a classical LCA to investigate the relative importance of episodic wet weather versus “continuous” dry weather loads coupled to further uncertainty analysis using a Monte Carlo method. Results analysis showed that a few severe events can be important contributors to the total annual pollutant load on some parameters (routine wastewater pollutants but also priority pollutants). The proposed method based on the definition and characterization of archetypical weather days has shown the appropriate level of temporal differentiation in the LCI to assess the impacts from unmanaged pollutant loads from UWS during intense storm events. With such significant contributions of pollutant loads at the LCIA level, further research is required to include temporally-differentiated emissions in the methodological framework of the aquatic categories of eutrophication and ecotoxicity, to better understand how the performance of an UWS system affects the receiving environment for given local weather conditions.

Innovative techniques for monitoring chemicals in the environment

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Analysis of 42 pesticides in fresh fish muscle by dispersive solid phase extraction with QuEChERS followed by LC-MS/MS analysis
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Multi-residue analytical methods for determination of pesticides in biota samples are scant in the literature. The present study describes a high sensitivity and selectivity method based on dispersive solid phase extraction with QuEChERS followed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis for determination of a range of different polarity pesticides in fresh fish muscle and its subsequent application to various real samples in order to, first, prove its suitability, and, secondly, have a first picture of the occurrence of the compounds investigated in the matrix under study. A total of 49 pesticides, belonging to the classes of organophosphorus, pyrethroids, carbamates, triazines, ureas and chloroacetamides, among others, were initially considered for analysis. Extraction of the pesticides from the biota samples (10 g) was performed with QuEChERS kits using acetonitrile (1:1 w/v), 4g $MgSO_4$, 1g NaCl, 1g NaCitrate and 0.5 g disodium citrate sesquihydrate, and subsequent clean-up with 400 mg PSA, 400 mg C18 and 1200 mg $MgSO_4$. LC was performed with a Purospher STAR RP-18e column (125 × 2 mm, 5 μm). MS/MS detection was carried out in the selected reaction monitoring (SRM) mode with a triple-quadrupole mass spectrometer equipped with an electrospray (ESI) interface working in both positive and negative ion modes along the analytical run. Quantification was performed by the isotope dilution method, based on the peak areas obtained for the analytes and their deuterated analogues. The performance of the analytical method was evaluated in terms of linearity, sensitivity, accuracy, matrix effects and precision, according to the guidelines set for pesticide residues analysis in food and feed (SANCO/12495/2011), with satisfactory results for 42 out of the 49 initially considered compounds. As regards sensitivity, most of the target compounds could be determined at levels below 10 ng/g. Application of the method to a set of real samples showed the presence of some of the target compounds in fish muscles at relatively low levels (in the ng/g range). Main advantages of the method are, in addition to high sensitivity and selectivity, simplicity, rapidity and relatively low cost. *Acknowledgement* - The authors thank the financial support of the EU (FP7 projects GLOBAQUA (265264) and SOLUTIONS (603437)) and the Generalitat de Catalunya (Consolidated Research Groups “2014 SGR 418 - Water and Soil Quality Unit” and 2014 SGR 291 - ICRA).

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Development of new analytical techniques for monitoring of POPs and emerging environmental contaminants
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Reliable and accurate analytical techniques are essential in order to study the occurrence, behavior, and fate of known and emerging environmental contaminants. The aim of this work was to develop a fast, simple, high-throughput analytical method for simultaneous determination of diverse persistent organic pollutants (POPs): carcinogenic PAHs, dioxin-like PCBs, and PBDEs and other flame retardants (FRs) along with diverse pesticides in a single analysis to improve the monitoring of these contaminants. Sample preparation for biological samples including fish (catfish, salmon, wreckfish) and shrimp was based on quick, easy, cheap, effective, rugged and safe (QuEChERS) extraction with acetonitrile followed by clean-up using dispersive solid phase extraction (d-SPE). The sample preparation is fast and inexpensive, where one analyst can prepare a batch of 10 pre-homogenized samples in one hour, using approximately $3 of materials per sample. The analysis entails fast low-pressure (LP) gas chromatography (GC) tandem triple quadrupole mass spectrometry (MS/MS) allowing separation of over 200 compounds and internal standards in 10 min. Satisfactory recoveries (70-120%) were achieved for the majority of analytes with relative standard deviations under 20% even at 1-5 ng/g spiking levels. To minimize sample size, and reduce amounts of organic solvents and internal standards added before the extraction, we evaluated variables affecting QuEChERS-based extraction yields of incurred contaminants in fish samples. Our results demonstrated that 2 g subsamples were adequate for analysis of the

incurred contaminants. Smaller test portion size often translates into faster, easier, and less wasteful methods, provided that the subsample adequately represents the original sample. To further improve identification of contaminants and to achieve lower detection limits, we are currently evaluating a new Agilent 7010 high sensitivity MS/MS. Also, novel lipid-removal materials and new sorbents were recently evaluated for the analysis of contaminants in salmon. The monitoring data on the occurrence of these contaminants may advance our understanding of their potential risk and aid in future risk assessment for the better protection of human health and the environment.

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Two-dimensional algal arrays as innovative biosensing tool

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Because of their rapidity and sensitivity, microalgal biosensors can be used as early warning systems to detect early effects on organisms exposed to chemical in environmental samples. Among all the biological endpoints explored in whole-cell biosensors, chlorophyll *a* fluorescence is known as a routine technique for the determination of the stress effect and reactive oxygen species (ROS) production is becoming a promising key marker to evaluate chemicals toxicity in the environment. However reproducible immobilization of the living cells in facile array format for biosensing is still challenging and their viability is difficult to maintain. In this context, alternative current (AC) dielectrophoresis (DEP) allows a rapid and reversible assembly of live cells from aqueous sample. In our study, an alternative current (AC) dielectrophoretic lab-on-chip setup was evaluated as a rapid tool to collect and assemble cells of microalga *Chlamydomonas reinhardtii* in two-dimensional (2D) arrays. Combined with fluorescence microscopy detection, the capability of using the 2D whole cell arrays to follow the algal sub-toxic response to several environmental contaminants, including mercury, methylmercury, copper, copper oxide nanoparticles (CuO-NPs), and diuron was explored. The results showed significant increase of the cellular ROS when *C. reinhardtii* was exposed to increasing concentrations of methylmercury, CuO-NPs and 10^{-5} M Cu, as revealed by enhancement of the CellROX® stain cells. Less sensitive to short term exposure to contaminants was algal autofluorescence, which decrease only upon exposure Hg, CuO-NPs and high concentrations of copper. Overall, this study demonstrates the potential of combining of AC-dielectrophoretically assembled two-dimensional algal array with fluorescence detection, alone or in combination with fluorescence stain, as a rapid and multiplex biosensing tool for environmental chemicals and toxins. Indeed, this tool allowed simultaneous monitoring of the temporal trends in cellular ROS generation and chlorophyll *a* fluorescence and significant results were detected in less than 2h.

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Quantification of emerging micropollutants in an amphipod crustacean by nanoliquid chromatography coupled with mass spectrometry in MRM3 mode

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Over the last decades, human activities (industries, agriculture, transports, consumption, urbanization…) and their impacts on the environment have not ceased to increase. Many molecules related to these activities are released to the environment and their occurrences within aquatic ecosystem become an important issue. In particular, the dose-response relationships of emerging pollutants on organisms at lower trophic levels remain little-known. In order to conduct biomonitoring studies, it is necessary to acquire knowledge about the kinetics of bioconcentration and depuration of these molecules within species, taking into account the inter-individual variability. In this context, an innovative analytical method was developed to quantify, in an amphipod crustacean (*Gammarus fossarum*), three emerging micropollutants known as anthropic pollution markers: carbamazepine, oxazepam and testosterone which are respectively an anticonvulsant, an anxiolytic and an endogenous hormone. A salting out liquid-liquid extraction, known as QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) was miniaturized and optimized. The miniaturization of the method made it possible to adapt to the low mass sample (approximatively 5 mg dry weight) and to perform the analysis at the scale of a single organism. Beside an efficient sample preparation, ultra-trace analysis of compounds in such matrices of small size requires a good sensitivity. Therefore, an original coupling was developed. It consisted of a separation by nanoliquid chromatography (nanoLC) coupled to tandem mass spectrometry using multiple reaction monitoring cubed mode (MRM^3). For the nanoLC separation, a preconcentration system by on-column trapping was optimized to increase the injection volume. In order to keep both sensitivity and selectivity, detection is usually performed by mass spectrometry in multiple reaction monitoring mode (MRM). Nevertheless, proteomic studies showed that sensitivity and selectivity could be improved using MRM^3 mode. This technic was developed in this project and compared to the classic MRM. In the best of our knowledge, this was the first time that $MRM^3$was used for small molecule (The analytical strategy developed allowed to obtain limits of quantification lower than 1 ng.g^{-1} (wet weight) and to establish the kinetics of bioconcentration of contaminants within *G. fossarum*.

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Time Integrating, Micro Flow, In-line Extraction (TIMFIE) sampler for determination of pesticide concentrations in water - a new quantitative tool in pollution monitoring

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There is a gap between the need for adequate concentration measurement of pesticides and other organic pollutants in water and the usefulness of the field sampling procedures available. In response to this a new device for quantitative, time integrated whole water sampling and simultaneous in-field extraction has been developed. The TIMFIE sampler is easily assembled from inexpensive laboratory consumables and requires no electricity or other supporting equipment. It consists of a simple syringe pump connected to a flow restrictor (PEEK capillary) that enables a low microliter per minute water flow through an in-line solid phase extraction (SPE) cartridge. The force that slowly fills the syringe is accomplished by attaching a rubber band or a weight to the piston, or through applying under pressure in the syringe by pulling and locking the piston prior to sampling. Target compounds are continuously extracted and trapped on the SPE material during the sampling period. The extracted water ends up in the syringe barrel and after finished sampling the sample volume is determined and the SPE cartridge sent to the analytical lab where it is handled according to standard protocols, typically followed by gas or liquid chromatography coupled to mass spectrometric detection. Method development and validation is similar to standard SPE procedures and the large number of SPE packing materials available together with the possibility of stacking cartridges in series offers flexibility. The flowrate, and in the end the sample volume, will mostly depend on the force applied, the diameter of the syringe and the diameter and length of the restrictor, but also on e.g. temperature since this affects water viscosity. By altering the controllable variables of the TIMFIE sampler the flow can be adjusted to enable sampling periods from hours up to weeks. The final volume collected during a certain time period will differ between sampling occasions, but the sampled/extracted water volume will always be determined with good accuracy, thus enabling quantitative analysis. The TIMFIE sampler offers:\n - Time-averaged sampling of whole water. \n - Quantitative determination of compound concentrations by well-defined procedures. \n - Low price, easy assembling and high flexibility. \n - Minimised costs for shipping and storage of samples. Experiences and results from lab and field experiments looking at pesticides in surface water will be presented.

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High throughput microbial array for complex matrix assessment

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Ecotoxicological assessment using different bioassays for environmental sample are largely used this last 20 years. Amoung them recombinant bioluminescent or fluorescent bacterial bioassays have been developed to determine the presence and the toxic effect of pollutants, because even if chemical analysis are sensitive and specific, they failed in the determination of the bioaccessibility and biodisponibility of the pollutant. All the recombinant bacteria carried out fusion between reporters genes (*lux* CDABE for bioluminescence and gfp for fluorescence) and the promoter of genes involved in metal resistance mechanisms (for metal detection) or genes involved in degradation of organic compounds. These bioassays are generally not specific for one compound, in particular those dedicated for Metal trace element (MTE), leading to a difficult application in complex media. In order to overcome this problem, we used an array of bioluminescent and fluorescent recombinant bacteria with different cross-sensitivity to MTE and organic pollutant. We combined the bacterial response of all the bacteria to obtained a database. In a frist approche we used a set of bioluminescent recombinant bacteria sensitive to MTE, we build a database with the results of the induction of the bacteria exposed to 15 metal and metal mixture. Four specific decision trees based on CHAID algorithm were designed and tested on waste water. The result showed that the concentrations of MTE detected by our model match the chemical measurements. In a second approche we completed our panel of bioluminescent bacteria to improve our model by using a collection of 1930 fluorescent bacteria. Prelimimary results obtained on complexe envrionmental samples (wood-waste leachates) showed variation of bacterial response according to the contamination allowing an interesting method of screening. We propose a mixture of targeted and non targeted highthroughput arrays of bacteria to describe more realistically the toxicity of very unusual complex matrix combined with data mining interpretation.

Biological effects of emerging micro pollutants at realistic

environmental concentrations (I)

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Non-steroidal anti-inflammatory drug diclofenac disturbs the prostaglandin system in the marine mussel Mytilus galloprovincialis

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Human pharmaceuticals, such as nonsteroidal anti-inflammatory drugs (NSAIDs), are an emerging environmental threat to marine organisms. Bioaccumulation in aquatic organisms along with possible ecotoxicological effects may then be of concern, as shown by the inclusion of diclofenac (DCF) in the first watch list by the EU Water Framework Directive. It is possible to hypothesize that these compounds will be pharmacologically active in organisms in which the drug targets are expressed and functional. In humans, NSAIDs act through inhibition of cyclooxygenase (COX) conversion of arachidonic acid into prostaglandins. PGs are conserved lipid signaling molecules that mediate a wide array of biological functions [1]. There have been many reports of PG biosynthesis in a broad range of invertebrates, including bivalves [2]. Therefore, modulation of PG production in marine bivalves following exposure to DCF is worth questioning. The objective of this study was thus to investigate whether diclofenac (DCF) affects PG levels in marine mussels *Mytilus galloprovincialis*. An experiment was carried out whereby marine mussels were exposed to DCF for 48 h to 1 and 100 μg/L DCF. A specific and sensitive analytical method using liquid chromatography tandem mass spectrometry was developed to quantify DCF accumulation in marine mussel tissues. The developed method could also clearly identify and quantify prostaglandins PGE_2, PGD_2 and $PGF_2\alpha$ levels in mussel tissue and be used to assess their modulation following DCF exposure. Measured concentrations of DCF in water were relatively close to the nominal concentrations for all exposed aquaria. A low bioconcentration was calculated at 26.4 L/kg. The weak bioaccumulation of DCF observed is consistent with its possible biotransformation in the organisms, supported by the detection of hydroxy-diclofenac metabolites in exposed organisms and water. Basal PGE_2 concentrations ranged from under the limit of detection (LoD) to 202 μg/kg dw. PGD_2 was always found below the LoD. A downward trend in the PGE_2 concentration was observed between non-exposed mussels and those exposed to 1 μg/L DCF, whereas this decrease was confirmed and statistically significant for exposure to 100 μg/L. PGF2a globally ranged from 90 to 518 μg/kg dw. No difference was observed for $PGF_2\alpha$ levels between controls and exposed organisms. [1] Tootle, T.L., 2013. Int. J. Biochem. Cell Biol. 45, 1629–1632. [2] Martinez, G. et al. 1999. J. Exp. Zool. 284, 225–231.

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Effects of Gadolinium-based contrast agents used in magnetic resonance imaging on freshwater mussels.

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Contrast agents based gadolinium (Gd-CA) are often used in Magnetic Resonance Imaging (MRI). After blood injection these compounds are released by urinary rejection. Due to the lack of specific recycling process in European Waste Water Treatment Plants (WWTPs), these pharmaceutical compounds enter the aquatic environment from river to tap water (Bau & Dulski, 1996). Until now, the effects of these compounds on living organisms has been approached in medical conditions i.e. 10^5 μg.L^{-1} range of concentration. During this study, toxicity and bioaccumulation of Gd-CA were followed at realistic environmental concentrations on two species of freshwater mussels (*Corbicula fluminea* and *Dreisseina bugdensis*). Gd concentrations and bioconcentration factors (BCF) were measured in mussels' tissues by ICP-MS analysis in bivalves exposed during 7 and 21 days : (i) *in situ* along a section of the Mosel River located around WWTP output and (ii) in laboratory in presence of the most currently used Gd-CA (Dotarem®) in realistic concentrations (1 and 10 μg.L-1) according to our previous studies (Perrat *et al.,submitted*). Moreover, ecotoxicological effects were evaluated via a battery of complementary biomarkers in mussels, i.e. catalase (CAT), caspase-3 (CSP-3), glutathione-S-transferase (GST), acid phosphatase (ACP), glutathione peroxidase (GPx), lactate dehydrogenase (LDH), lipid peroxidation (LOOH), electron transport system attached to the mitochondria (ETS), and the total antioxidant capacity (TAC). Our results showed an increase of Gd concentrations in the digestive gland of mussels after 21 days of exposure; however Gd-CA is less accumulated by *Corbicula fluminea* (BCF = 11) than *Dreisseina bugdensis* (BCF = 20). In laboratory, no mortality was observed on bivalves. Biomarkers measurements showed an impact of Gd-CA on bivalves suggesting establishment of detoxification mechanisms in *Corbicula fluminea* tissues; e.g. an increasing of TAC level for *Dreisseina bugdensis* after 7 days of exposure. Gd-CA exposition seems to induce antioxidant mechanisms to protect the organism (Doherty *et al*., 1987). This study presents ecotoxicological effects and accumulation of Gd in bivalves in realistic conditions of exposure to Gd-CA. To go further, we will implement bioaccumulation and toxicity assays with other aquatic organisms and effects along a short trophic chain (algae-daphnids-fish).

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The marine mussel Mytilus galloprovincialis bioconcentrate and metabolize antidepressant pharmaceutical venlafaxine

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A significant investment in research has been conducted to define exposures and potential hazards of pharmaceuticals in freshwater and terrestrial ecosystems. In contrast, comparatively few empirical studies have been conducted for pharmaceuticals that are likely to enter coastal and marine ecosystems[1,2]. The antidepressant venlafaxine (VLF) and some of its metabolites were recently found in mussels caged in a coastal site receiving treated wastewater[3]. In those marine organisms, very scarce data is available on the accumulation and/or metabolisation of pharmaceuticals. Consequently, it appears hazardous to conclude on the origin of those metabolites in mussels, which could include a bioaccumulation through direct exposure as well as a metabolism of VLF in mussels. The aim of the present work was to quantify the accumulation of VLF in the marine mussel Mytilus galloprovincialis and to evaluate the possible metabolism in laboratory controlled experiments. The accumulation of VLF was evaluated in the whole mussel tissues after 1, 3 and 7 days of semi-static exposure by water (10μg L-1day-1) followed by 1, 3 and 7 days of depuration. Under those conditions, VLF attained an average tissue concentration of (n=3) 2146.3 ± 156.0 ng g-1 dry weight (d.w.) in seven days. The kinetic bioconcentration factor (BCF) was 265 ± 19 L kg-1 d.w. Seven days of depuration allowed a decrease of tissue concentration to 21 ± 1.0 ng g-1 d.w. Four VLF metabolites were quantified in mussel tissues and excreted in water. The kinetics of those metabolites in water confirmed the metabolism of VLF by mussels. Complementary experiment conducted at 1, 10 and 100 μL L-1 nominal concentration clearly confirmed that M. galloprovincialis metabolize VLF, with the quantification of studied metabolites excepted the NNO-VLF. These results gave a first approach on the ability of mussel to metabolize pharmaceuticals. Together with bioaccumulation information, this study provided a first approach on the pharmacokinetics of a pharmaceutical in a wild marine species, underlining the need of further experiments to better understand how venlafaxine modulate the receptors in mussels and how exposure to this antidepressant affects physiological functions of invertebrates. [1] Arpin-Pont, L., ESPR. Doi : 10.1007/s11356-014-3617-x. [2] Gaw, S., Philos Trans R Soc Lond B Biol Sci. 2014; 369(1656): 20130572. [3] Martinez Bueno, M.J., Anal Bioanal Chem 2014; 406:601–610.

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Mechanism study for cardio negative effect of propranolol to Daphnia magna by utilizing acute toxicity syndrome

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Acute toxicity syndrome is a set of biological responses in behavioral, physiological parameters resulted from distinguishable toxic mechanism. In aquatic toxicology field, monitoring study for the syndromes has been conducted to fish and has proven ability to categorize chemical agents based on their mechanism of action because different molecular stimulation result in different visible outcomes. In this study, we tried to distinguish mode of action of propranolol, a human pharmaceutical ingredient, which is usually considered as a baseline toxic chemical to aquatic non-targeted species based on lethal concentration from QSAR. *Daphnia magna* was employed as a model organism and not only phenotypical outcomes but also metabolomics profiling were monitored for more stereoscopic insight in mechanism characterization. Narcotic agents (4-chloroanilin, ethylacetate), β-adrenergic receptor and 5-hydroxytryptamine receptor antagonists (nadolol, yohimbine, cyproheptadine) were treated with propranolol to *Daphnia magna* and their syndromes were compared to categorize potential mechanism of propranolol. Syndromes include swimming behavior, heartbeat and metabolite profiling. In the time series monitoring during 24h of swimming behavior, *Daphnia magna* tend to be deactivated at high concentration of propranolol. Likewise, nadolol and yohimbine showed hypo activity as time goes by in effective concentrations. In opposite, exposure of cyproheptadine resulted in hyper activity and narcotic agents showed recovery in swimming activity after severe temporary deactivation. Activity-concentration relationship was compared between swimming behavior and heartbeat at 6h. Although propranolol exposure affect to both swimming behavior and heartbeat, heartbeat was decreased at concentrations where no effect on swimming behavior was observed. Most similar pattern was observed from nadolol exposure and yohimbine also showed similar relationship with higher effect on swimming activity. For 4-chloroaniline and ethylacetate, swimming behavior was so sensitive that several concentrations showed deactivation without significant heartbeat effect. NMR Analysis is now being conducted for metabolite profiling and the result will be combined and be presented. We expect that metabolite profiling provide information how metabolism balance changed with chemical exposure and stricter categorization tool by integration with visible outcomes described above.

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Environmentally relevant concentrations of antidepressants alter behaviour and gene expression in amphipods

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The effects of antidepressants on wildlife are currently raising some concern due to an increased number of publications indicating biological effects at environmentally relevant concentrations (< 100ng/L). These results have been met with some scepticism due to the higher concentrations required to detect effects in some species and the perceived slowness to therapeutic effects recorded in humans and other vertebrates. Since their mode of action is thought to be by modulation of the neurotransmitters serotonin, dopamine, and norepinephrine, aquatic invertebrates that possess transporters and receptors sensitive to activation by these pharmaceuticals are potentially affected by them. We highlight studies on the effects of antidepressants, on particularly crustacean groups showing they are susceptible to a wide variety of neuroendocrine disruption at environmentally relevant concentrations (< 100ng/L). Behavioural and transcriptional changes in this crustacean were studied when exposed to the most prescribed SSRIs (citalopram, sertraline and fluoxetine), SARI (trazodone) and SNRI (duloxetine). The animals were exposed to these five drugs at environmentally relevant concentrations from 0.001 to 1 μg/L during short-term (1 hour and 1 day) and medium-term (8 days) experiments. The movement of the amphipods was tracked using the behavioural analysis software during 12 min alternating dark/light conditions. Antidepressant concentration had a significant effect ($p < 0.01$) on velocity for Duloxetine (1hr, 1day & 8days); Sertraline (1hr & 1day) and Fluoxetine (1day) but not trazodone or citalopram ($p > 0.05$). We have also applied high-throughput sequencing technology to animals exposed to 100ng/L concentrations of sertraline, fluoxetine and duloxetine to reveal the broad transcriptomic responses to these compounds. Preliminary analysis of gene expression profiles indicates a broad range of potential neurological pathways confirming that these drugs have multiple targets. In the light of new studies indicating effects on the human brain with just of dose of SSRIs using MRI scans, we discuss possible reasons for the discrepancy in former results in relation to the "read-across" hypothesis.

Ecotoxicology and risk assessment of nanomaterials - Interactions at nano-bio interface (II)

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Eco-interactions of engineered nanoparticles in sea water media and implications for toxicity

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The physico-chemical properties of engineered nanoparticles (NPs) such as specific surface charges might play a crucial role in their behavior in complex natural media as sea water, with implications on cellular uptake and toxicity in exposed organisms. In this study, we investigate the ecotoxicity of nano-TiO_2 (25 nm), PS-COOH (40 nm) and nano-SiO_2 (100 nm) as model of negatively charged NPs with different size and inner core in order to evaluate their suitability for NPs screening based on their interactions with natural sea water media (NSW). Marine green microalgae *Dunaliella tertiolecta* and brine shrimp *Artemia franciscana* were used as model species in standardized acute (72h and 48h) and long-term (7d) ecotoxicity tests. A detailed physico-chemical characterization of all three NPs in NSW and in comparison to artificial sea water (ASW) and Milli-Q water (Milli-Q) was performed. Given their common negative surface charge, the effect of abundant background bivalent and monovalent counter ions in NSW on the suppression of electric double layer was dramatic and lead to strong aggregation. HR-TEM of nano-TiO_2 suspended in NSW revealed a complex matrix around aggregates, forming corona-like structures (eco-corona), further confirmed by DCS analysis. Ecotoxicity tests showed a common pattern of no toxicity for all three NPs in the range of the tested concentrations (0.1-100 mg L^{-1}) for both microalgae and brine shrimps. Nano-TiO_2 and PS-COOH resulted massively sequestered inside the gut lumen of brine shrimp larvae while nano-SiO_2 aggregates were barely detectable probably due to their optical transparency. Our findings suggest that negative surface charges drive substantial aggregation of NPs in NSW thus limiting uptake and consequently toxicity in exposed organisms. Therefore ecotoxicity coupled with a detailed physico-chemical characterization in the suspension media might be used as suitable tool to predict behavior and potential adverse effects of NPs in the marine environment.

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Are trophic interactions between aquatic invertebrates affected by AgNPs exposure route?

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Silver nanoparticles (AgNPs) have been increasingly used over the last decade, resulting in their growing release into freshwater ecosystems, where AgNPs and ionic Ag derived from NPs can have toxic effects on aquatic species and compromise important processes such as organic matter use and decomposition. However, little is known about how species interactions in multi-trophic systems will affect such ecosystem processes when exposure routes of AgNPs vary. The goal of this study was to assess the impacts of low doses of AgNPs and Ag^+ on trophic interactions between aquatic invertebrates and consequent ecosystem effects. Specifically, we assessed the importance of both direct (via water) and indirect (via food) exposure routes by exposing a simplified detrital food web, comprising leaf litter, microbes, a shredder species and collector species to: (i) water contaminated with AgNPs (0, 1, 100 $\mu g\ L^{-1}$) or $AgNO_3$ (0, 0.1 $\mu g\ L^{-1}$), and (ii) leaves contaminated for 6 days with AgNPs and $AgNO_3$ at the same concentrations. Shredders (*Gammarus pulex*, Gammaridae, Amphipoda) and collectors (*Habrophlebiodes sp*., Leptophlebiidae, Ephemeroptera) were placed in laboratory feeding containers where collectors had access to fine particulate organic matter (FPOM) produced by *G. pulex*, but were separated by a mesh screen. Leaf consumption and FPOM production by *G. pulex* as well as fungal biomass and decomposition activity were determined after 15 days. The stress induced by AgNPs and Ag^+ in both invertebrate species was assessed by measuring the activity of antioxidant and neuronal enzymes. Microbial decomposition was lower by direct exposure to AgNPs and Ag^+, whereas leaf consumption by *G. pulex* only decreased when leaves were contaminated with the lowest concentration of AgNPs. There were no effects on FPOM production. Changes in the activity of a key antioxidant enzyme, catalase, indicated stress caused by AgNPs and Ag^+ in both invertebrate species, mainly in response to direct exposure. Overall our results demonstrate that ecological effects on different functional groups of stream invertebrates vary with exposure route of AgNP at environmentally realistic concentrations. Thus the pathway by which stream food webs are exposed to AgNPs will influence the impacts of AgNPs on food-web components and the processes in which they are involved.

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Food chain transfer of silver nanowires in aquatic ecosystems

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Nanowires, which are increasingly being used in various industries, may have different adverse ecological effects from those of zero-dimensional nanoparticles, which have been widely studied. We investigated the direct and indirect (trophic) effects of two sizes (10- and 20-μm long) of silver nanowires (AgNWs) on a three-species aquatic food chain consisting of algae (*Chlamydomonas reinhardtii*), water fleas (*Daphnia magna*) that feed on algae, and their predators zebrafish (*Danio rerio*). We assessed the direct toxicity of AgNWs for algae and water fleas, and documented the transfer of AgNWs absorbed by algae to water fleas fed with exposed algae, and eventually to zebrafish fed with exposed water fleas. We found that AgNWs directly inhibit growth in algae, and destroy the digestive organs of water fleas. We also found that AgNWs can be transferred through food chains, and can enter and impact the bodies of organisms in higher trophic levels, such as the fish in this study, implying that nanomaterials have the potential to eventually enter human diet. We emphasize the need for research that takes into account environmental factors, food web complexity, and nanomaterial diversity, in order to better understand the role of nanomaterials in affecting natural communities as well as human health. *Acknowledgement - This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (2013R1A1A2061386), and the Ministry of Science, ICT and Future Planning (2014R1A2A1A11050513).*

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Al2O3 nanoparticles reduce toxic effects of thiacloprid on the non-biting midge Chironomus riparius

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The increasing number of publications in the last years show that nanoparticles become more a topic of high concern in ecotoxicology. This is, on one hand, due to increasing usage of nanomaterials while, on the other hand, little is known about their fate and interactions in the environment. Hence, we wanted to examine, if and how Al_2O_3 nanoparticles interact with the neonicotinoid insecticide thiacloprid. To test this, we measured how toxicity of thiacloprid is altered by Al_2O_3 nanoparticles, based on the mortality rate of the non-biting midge *Chironomus riparius*, a well-established study organism in ecotoxicology. The conducted acute toxicity test with fourth instar larvae of *C. riparius* revealed that Al_2O_3 nanoparticles as single substance had no effect on the mortality rate. However, larvae which were exposed to a mixture of thiacloprid and nanoparticles showed a decreased mortality rate, in relation to larvae that were exposed to thiacloprid solely. This protective effect of the Al_2O_3 nanoparticles was inversely correlated with the applied amount of nanoparticles, with higher concentrations of Al_2O_3 nanoparticles resulting in lower mortality. The underlying mechanisms of these interesting phenomena are still under investigation. Yet, solely sorption of thiacloprid onto nanoparticles (pristine and aged) can be excluded as underlying mechanism. Further research will focus on chemical analyses, histopathology as well as an extendend exposure regime of the larvae, which is based on the OECD guideline 218.

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Anthracene sorption to TiO2 nanoparticles and bioavailability of UV-activated anthracene by-products in larval zebrafish.
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Engineered nanoparticles (NPs) can be released into aquatic environments and present a risk of toxicity for aquatic organisms. Among the most commonly used NPs are TiO_2-NPs, and, although these NPs appear to be of minimal toxicity, their potential for photo-activity and sorption to toxic substances are persisting environmental concerns. Several studies have shown sorption of organic compounds to TiO_2-NPs and enhanced toxicity of the co-contaminants. The toxicity of polycyclic aromatic hydrocarbons (PAHs) has been extensively studied and there is information on the toxicity of UV-activated PAHs but the interaction between TiO_2-NPs, PAH sorption, and UV-activation is unknown. The objective of this study was to evaluate sorption of anthracene to TiO_2-NPs by 1) analysis of the preparations with and without UVA irradiance by fluorescence spectroscopy and 2) assessment of changes in expression of target biomarker genes including cytochrome P450 1A (*cyp1A*), DNA repair (*ddb2*), superoxide dismutase (*sod1*) and aryl hydrocarbon receptor 2 (*ahr2*) genes, in larval zebrafish. Zebrafish larvae (72 hpf) were exposed (24 h) to anthracene (0-30 μg/L) in freshwater ([Ca^{2+}] = 71 mg/L according to OECD no. 203). Preparations were exposed to 80 kJ/m^2 UVA at the end of the 24 h exposure, and larvae were sampled at 3 h after UVA exposure. For experiments in which sorption of anthracene to TiO_2-NPs (4-8 nm diameter) was investigated, TiO_2-NPs (2 mg/L) were added to anthracene preparations and stirred for 24 h prior start of exposure. Anthracene was not acutely toxic to zebrafish larvae at the concentrations tested. After the UVA exposure, zebrafish mortality was 28±8% (±SD, n=3) at the highest anthracene concentration (30 μg/L). When fish were exposed to anthracene and UVA, a 45-fold increase in *cyp1A* expression and a 1.7 fold-induction of *sod1* were observed at 15 μg/L anthracene. The presence of 2 mg/L TiO_2-NPs with anthracene resulted in no significant change in expression of either *cyp1A* or *sod1* after UVA exposure compared to unexposed controls. For fish exposed to TiO_2 (2mg/L) and UVA without anthracene there was no induction of either *cyp1A* or *sod1*, which is in accordance with other studies that have also not observed photo-induced toxicity at relatively low TiO_2-NP concentrations. Future work will investigate the association between TiO_2-NPs and PAHs and the products of photo-induction.

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Influence of multiwalled carbon nanotubes on the toxicity and bioaccumulation of triclocarban in aquatic organisms of different trophic levels
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Although the acute toxicity of MWCNTs is rather low, this nanomaterial was shown to be taken up by different organisms and could therefore influence the bioavailability of environmental pollutants due to its high sorptive capacity. We investigated the sorption of the antibacterial and preservative agent used in personal care products, triclocarban (TCC), to MWCNTs, and the influence of this interaction on the bioavailability of TCC for organisms related to one another in the food chain: the green alga *Desmodesmus subspicatus*, the water flea *Daphia magna*, the brine shrimp *Artemia salina*, and the zebrafish *Danio rerio*. Next to unaltered MWCNTs, the released material of plastic composites, in which MWCNTs were embedded, was tested. It was shown that the presence of MWCNTs protects the polymer matrix from degradation due to simulated solar radiation. However, up to 1% of the embedded CNTs were released from irradiated samples that were transferred to different media, whereas release from non-irradiated control samples was much lower. Both single nanotubes as well as nano- and microscale CNTs-polymer fragments were detected and visualized. Both *D. magna* and *A. salina* were able to take up this material. TCC sorption to MWCNTs was quite high with sorption coefficients ($LogK_{CNT}$) of about 7.5 in all media. This resulted in lower toxicity of TCC to all test organisms when MWCNTs were present in the exposure media. Similarly, higher bioaccumulation factors (BAFs) were obtained when MWCNTs were absent in the media. These results show that MWCNTs lower the bioavailability of TCC to freshwater and saltwater species although this nanomaterial is known to be taken up by these organisms to a relatively large extent. From the elimination experiments with *D. magna* and *A. salina*, it can be derived that excretion of TCC was faster when MWCNTs were present in the media during the uptake phase. Hence, it seems that TCC bound to MWCNTs is hindered from passing the gut epithelium. Currently, biomagnification experiments are performed in order to evaluate the importance of food chain transfer of MWCNTs, TCC, and TCC-CNTs complexes. Moreover, it will be determined whether the organic compound and the nanomaterial are distributed to different organs and tissues of fish after dietary exposure.

Microplastics in the environment: Sources, Fate and Effects (IV)

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On the potential role of phytoplankton aggregates in microplastic sedimentation
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Microplastics (MPs) pollution is now a major concern in ecotoxicological topics. To better assess the impact of MPs on marine environment, scientists need to understand MPs cycle. Recent estimations of small size MPs concentrations at the surface layer of the oceans were lower than model-predicted estimations based on larger plastic wastes. Different sinks were suggested to explain this mismatch including physical mechanisms and interactions with marine organisms. Among marine organisms, phytoplankton may have a role in MPs distribution. In this experiment, we studied the possibility for phytoplankton aggregates to bind and incorporate MPs through their sedimentation. At the same time we investigated the respective impacts of MPs on aggregate parameters and the impacts of aggregates on MPs sink. Three types of phytoplankton aggregates (*Chaetoceros* neogracile aggregates, *Rhodomonas salina* aggregates and a mix of the two species) were produced in a roller-tank. These aggregates were exposed to a flow (680 mL per hour) of 2 μm microbeads of polystyrene (yellow-green fluorescent, 1.050 kg L^{-1}, 10^4 beads mL^{-1}) in a roller tank that mimic aggregates sink. After exposure, aggregate parameters were assessed like size, permeability, excess density, sinking rates and MPs content. Diatom aggregates were on average bigger, stronger, stickier and sunk faster than the aggregates made from *R. salina* or from the mix of microalgae. After MPs exposure, all laboratory-made aggregates incorporated MPs. The smaller and more fragile aggregates from *R. salina* incorporated more MPs than *C. neogracile* and mix aggregates. When incorporated into aggregates, sinking rates of MPs increased from tenths to several hundred meters per day. Simultaneously aggregates sinking rates was impacted. Sinking rates of *C. neogracile* aggregates strongly decreased while sinking rates of *R. salina* increased. This study demonstrated that MPs can be incorporated through aggregates during their settling in the water column, and that the efficiency depends on the composition of marine aggregates. A new pathway was highlighted for MPs sink that may partially explain the lack of MPs in surface layer. Even though further studies are needed with different laboratory-made aggregates or field sampled aggregates to better assess the potential for marine snow to aggregate MPs. In parallel, *in-situ* quantification of MPs in marine snow from should be done to address the relevance of this process in MPs cycle.

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Aquatic ecotoxicity testing of nanoplastics - lessons learned from nanoecotoxicology

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Micron-sized particles have so far been the main research focus in regard to aquatic plastic particle pollution. Due to mesh netting sizes, nanoplastics are not detected in commonly used methods for plastic pollution surveys. Nonetheless, nanoplastics are likely to be pervasive in the aquatic environment. Here, as for microplastics, nanoplastics may cause direct ecotoxic effects and carrier-effects through adsorption of co-contaminants and the smaller particle size could be an influential factor. However, actual knowledge is currently limited to a few scientific studies. Based on literature information, combined with our own previous work on nano- and microplastics as well as test method development and adaptation for ecotoxicity testing of engineered nanomaterials, we here present an overview of lessons learned from nanoecotoxicology. We offer suggestions on how these can be transferred into recommendations for ecotoxicity testing of nano(and micro)plastics. In addition we suggest how to implement some of these recommendations in algae growth inhibition tests, studies of carrier-potential of nanoplastics and uptake in aquatic invertebrates and fish.

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Effects of ingested PVC micro particles with and without sorbed benzo(a)pyrene on the cellular and sub cellular processes of the macrobenthic organism Hediste diversicolor
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Along with impacts such as global warming, ocean acidification and habitat destruction, pollution can cause undesirable changes in marine ecosystems. In the last decade, plastic pollution has been a matter of increasing scientific and public concern. Marine debris is comprised primarily of non-biodegradable plastic polymers and it is considered a multiple stressor in aquatic habitats as a consequence of the large mixture of chemical contaminants potentially adsorbed from the aquatic environment and/or associated with it in the polymer production. Polymers with densities higher than seawater (i.e., PVC) tend to sink and continuously accumulate in sediments. Animals from sedimentary habitats are therefore vulnerable as microplastic particles may adsorb organic pollutants from the aquatic environment and be largely accumulated in sediments. In laboratory conditions, microplastics have been shown to be ingested by amphipods, barnacles, mussels and lugworms [1]. However, the available data concerning the effects of microplastic particles is limited and even less is known concerning the “Trojan horse” effect caused by microplastics contaminated with ubiquitous and highly toxic environmental contaminants such as PAHs. Thus, the current study focused on the acute effects of the combined exposure to microplastics and benzo(a)pyrene sorbed particles on the macrobenthonic model organisms *Hediste diversicolor*, a species with a relevant role in trophic webs.

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A trophic food web experiment with Artemia nauplii and zebrafish (Danio rerio) for the transfer of microplastics and associated POPs
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In the present study, a simple artificial food chain with *Artemia* spec. nauplii and zebrafish (*Danio rerio*) was established to analyze the transfer of microplastic particles and associated persistent organic pollutants (POPs) between different trophic levels. The uptake of microplastic particles and the transfer of potential harmful substances along with microplastics has been studied in a variety of organisms, especially invertebrates. However, the potential accumulation of very small microplastic particles along food webs ending with vertebrate models has not been studied so far. *Artemia* nauplii and zebrafish were exposed to very small (1 - 20 μm) microplastic particles, which accumulated within nauplii and were subsequently transferred to fish. For the analyses of the transfer of POPs *via* microplastics, the polycyclic aromatic hydrocarbon (PAH) benzo[a]pyrene (BaP) was used since (a) it is a common POP also found on environmental microplastics and (b) it shows autofluorescence at respective wavelengths. Virgin particles not loaded with BaP did not cause any observable physical harm in the intestinal tracts of zebrafish. The visual fluorescence tracking of BaP was strong enough to detect the transfer and accumulation of BaP both in *Artemia* nauplii and zebrafish intestines. This indicates that food-borne microplastic-associated POPs do desorb in the intestine of fish and are thus transferred to the intestinal epithelium and liver of higher organisms. However, further research is needed on different spectra analyses and metabolization of microplastic-associated POPs.

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Does microplastic in recycled organic resources pose a risk to terrestrial environments?
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Problems related to plastic litter pollution have been recognised since early 1970’s, and in recent years microplastic (MP) contamination of particularly the marine environment has been subject to increased focus. Although a number of publications in the last couple of years have pinpointed that also freshwater systems may be at risk from MPs, mainly due to release of particles from waste-water (WW) treatment processes, MP contamination of terrestrial environments has up until now rarely been studied. Due to processes taking place in WW treatment plants, a large proportion of particles in WW is likely to settle out in the sludge together with organic material. This reduces the amount of MPs in the effluent water and consequently contamination of aquatic environments. However, when sludge resulting from WW treatment processes is used for soil amendment, in order to recycle phosphate for agricultural purposes, deposited plastics are added to the terrestrial environment. Because plastic is slowly broken down it may accumulate in soils. To assess potential risk of MPs in terrestrial environments it is necessary to increase knowledge on type and amount of MPs in organic resources as well as in recipient soils. At the same time studies of potential effects of MP to soil organisms is largely absent from the scientific literature. In this presentation we give an introduction to what we currently know about potential exposure in terrestrial environments, including some recent studies from our lab on type and amount of MPs in Danish WW sludge and soils fertilised with different organic resources. These studies confirm the presence of MPs in both sludge and soils fertilised with sludge or composted household waste. We also include preliminary information on hazard of MPs in terrestrial environments, based on recent experiments on epigeic earthworms, studying the effects of MPs alone and in combination with other environmentally relevant contaminants. The first studies showed limited effects of MPs on earthworms, which however may be due to the employed exposure scenario. Results will be compared to on-going studies with a modified exposure scenario. Although studies so far suggest that MPs will end up in the terrestrial environment, knowledge on hazard of MPs for terrestrial organisms is currently limited as are more ecologically relevant exposure predictions. Therefore more knowledge is required to realistically assess the risk of MPs in terrestrial environments.

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Be positive or negative? Long-term toxicity of polystyrene nanoparticles to marine planktonic species
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Micro as well as nanoplastics have been identified by the international community as one of the greatest challenges for marine ecosystems worldwide. Concerning the nano-sized debris (< 1 μm), their occurrence in the environment has not been quantified yet, due to current standardized methods for sampling as well as identification by analytical techniques. Polystyrene nanoparticles (PS NPs) have been recently adopted as model for nanoplastics in short-term ecotoxicological studies, but data concerning long-term toxicity on marine organisms are still lacking. Here we report the effects of nanoplastics to two marine planktonic species as green microalga *Dunaliella tertiolecta* and brine shrimp *Artemia franciscana*, exposed to anionic carboxylated (PS-COOH) and cationic amino (PS-NH_2) PS NPs. Since aggregation and surface charges can be key factors driving the eco-interactions of nanoplastics in the marine environment, their behaviour was assessed by DLS in both natural sea water (NSW) and algal medium (AM) as well as the formation of an eco-corona investigated. In terms of ecotoxicity, PS-COOH did not affect the growth of marine algae (72 h) and brine shrimps (14 d) up to 25 μg/ml and 10 μg/ml respectively but high retention of PS-COOH aggregates was observed in the digestive tract of brine shrimps at 14 days. On the opposite, PS-NH_2 caused high mortality of brine shrimps (LC_{50} 0.83 μg/ml, 14 days) as well as inhibition of algal growth and alterations (at 5 μg/ml) in the microalgae plasma membrane respect to the control group. These results represent a first insight into nanoplastics long-term toxicity to marine planktonic species underlining the role of nanoplastics surface charge in agreement with previous findings on other invertebrate marine species. This study emphasizes that long-term exposure constitutes a valid tool to assess the ecotoxicity of emerging contaminants including nanoplastics.

State of the science on poly- and perfluoroalkyl substances (PFASs) in the environment and humans (I)

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Characterizing spatiotemporal pattern of global PFAS releases using a dynamic substance flow analysis model
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This work developed a dynamic substance flow analysis (DSFA) model to imitate the temporal evolution and spatial shift of global environmental releases of homologous fluorotelomers and perfluorocarboxylic acids from both lifecycle and end-of-life stages. Major findings include: (i) The temporal pattern is illustrated by the evolution of environmental releases of PFOA and 8:2FTOH in China. Our DSFA results show that industrial processes are predominant sources to PFOA releases, therefore the scheduled phase-out of PFOA is anticipated to nearly eliminate future PFOA releases, while industrial processes and end-of-life disposal have almost equal contributions to 8:2FTOH releases. The time course of environmental releases of 8:2FTOH exhibit two sequential peaks a ~10-year gap, which indicates that end-of-life emissions from waste stocks will be noticeable sources in the future. (ii) The spatial pattern is demonstrated using snapshots of geographical distribution of global PFOA and 8:2FTOH emission hotspots for 1995, 2010 and 2020. The emission hotspot of PFOA shifted from Europe and North America in 1995 to China in 2010. The evolution of 8:2FTOH emissions in China resembles that in Europe and North America but with a ~10-year delay. This delay means that the West Pacific would become a remaining dominant source region in 2020 if no appropriate end-of-life managements are implemented. Our case highlights the implication and importance of the DSFA in future risk management of PFASs. Based on our DSFA-derived emission estimates, scientists are able to appraise the performances of future end-of-life management options and assess the impacts of the geographical shift of emissions on environment and human.

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Assessing the temporal shift of concentrations of perfluorooctane sulfonic acid (PFOS) in the environment following industrial transition

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Long-chain perfluoroalkane sulfonic acids (PFSAs), including perfluorooctane sulfonic acid (PFOS), are a group of chemicals that have been widely used since the late 1950s. These substances are highly persistent, bioaccumulative, toxic and are distributed ubiquitously in the environment, biota and humans. In 2009, Paul et al. and Armitage et al. estimated the global emissions of PFOS in 1970–2012 and in 1957–2030, respectively. Armitage et al. coupled their emission inventory model to a global-scale multimedia mass-balance model to assess the global fate and transport of PFOS and PFOS precursors (PreFOS). However, they note that their modeled atmospheric PreFOS concentrations were too low by one to two orders of magnitude compared to measured concentrations. Furthermore, there has been an ongoing global industrial transition that was not fully characterized in previous studies. The year 2000 was the start of a voluntarily phase out and regulatory restrictions of PFOS production and use in western countries. In contrast, manufacturers in China have reported large increases in their production. Increased use of PreFOS based insecticides in Brazil and neighboring countries has also taken place. In this study, we redesign and establish a new global emissions inventory of PFOS and PreFOS based on up to date information. We estimate between 1400-8400 tonnes and 2200-4100 tonnes of PreFOS and PFOS, respectively, to have been emitted between 1958 and 2015. These results are higher than those estimated by Armitage et al. (735-4005 tonnes PreFOS and 285-2565 tonnes PFOS) due to consideration of more detailed manufacturing emissions data, direct use of PreFOS as ingredients in products, and the degradation of complex molecular substances into PreFOS. To get an improved overview of transport and fate in the environment during this transition period, we combine this new inventory with a global-scale multimedia mass-balance model to derive environmental concentrations that we then compare to measured concentrations. A decline following the industrial transition period is visible within two to three years in the atmospheric concentrations of xFOSAs and xFOSEs, whereas a longer delay of five to six years exists before an impact is seen on the modeled PFOS concentrations in oceans.

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Levels of perfluoroalkyl substances in global air from 3 years of sampling under the Global Atmospheric Passive Sampling (GAPS) Network

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Poly- and perfluoroalkyl substances (PFASs) have been used globally with commercial and industrial applications that include oil and water repelants, and film-forming fire fighting foams. Perfluorooctane sulfonic acid (PFOS) is of particular interest as it is a priority chemical under the United Nations Environment Program (UNEP) Stockholm Convention for Persistent Organic Pollutants (POPs). The Global Atmospheric Passive Sampling (GAPS) Network monitors priority chemicals at over 50 global sites, and is the only global-scale air surveillance network measuring PFASs in air. PFASs were monitored at GAPS sites using Sorbent Impregnated Polyurethane Foam (SIP) disk passive air samplers (PAS) during three sampling years, to determine spatial and temporal trends of PFASs in global air. The data presented here shows the first global air comparison of PFASs, using SIP disk PAS samplers. Perfluorobutanoic acid (PFBA) had the highest concentrations in all samples (3) and was detected at almost every site sampled during the 3 campaigns. Temporal trends were not seen for any PFAS in sampled sites and spatial trends were not observed for any of the perfluorosulfonic acids. However, PFBA showed evidence of reduced levels at Asia and African sampling sites as compared to levels seen in Europe and Central/North America. PFOS had the highest concentrations of the sulfonic acids (3) and a reduction in global air concentrations of this chemical due to the phase out of PFOS in 2009 is not yet in evidence.

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Effects of weathering on PFASs used in durable water repellence of textiles

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Per- and polyfluoroalkyl substances (PFASs) are used in textiles for their oil and water repellent properties. Because PFASs with long perfluorinated chains have been shown to be persistent in the environment, bioaccumulative and (eco)toxic, the textile industry is phasing-out the long-chain PFASs and is replacing those compounds with alternative chemistries to deliver the desired durable water and soil repellent (DWR) effect. Those alternative chemistries can be divided in three main groups: fluorocarbon-based, silicon-based and hydrocarbon-based polymers. In the SUPFES (Substitution in practice of prioritised fluorinated compounds for textile applications) project the alternative DWRs are assessed to (i) their structural properties and connected performance, (ii) loss and degradation processes resulting in diffuse environmental emissions, and (iii) hazard profile for the emitted substances. As part of SUPFES weathering experiments are performed with textiles from outdoor clothing which are exposed to elevated UV radiation, humidity, and temperature in an aging device for 300 h, which is equivalent to the life time of the outdoor clothing. Before and after aging of the samples, the textiles were analysed for their perfluoroalkyl acid (PFAA) content. Results showed that weather conditions, like sunlight, high temperature, or humidity have an effect on PFASs used in DWR of outdoor clothing. Concentration of PFAAs increased, and PFAAs not present in the original textiles were formed during exposure to weather conditions. More research is needed to clarify the origin of the PFAAs and to determine the transformation route.

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Investigation on the presence and behaviour of precursors to perfluorinated compounds in the environment

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Wastewater treatment plants (WWTPs) have been identified as a significant pathway for the introduction perfluoroalkyl and polyfluoroalkyl substances (PFASs) to natural waters. In several studies it was observed that the concentration of certain PFASs were higher in the WWTP effluent compared to the corresponding influent. One reason for this might be the biotransformation of precursor substances, which were converted into known and analysed PFASs. The objective of the present study was the identification of potential precursor substances of persistent PFASs in WWTPs. Following the development of robust and validated analytical methods 65 PFASs, which included persistent PFASs and their potential precursors, sampling campaigns in six WWTPs (industrial and municipal, located in Europe) over a period of four weeks each were realised. The influent water and the effluent water, the air above the influent and the sludge of the WWTPs were sampled and analysed. Several transformation products of fluorotelomer alcohols (FTOHs) e.g. the unsaturated fluorotelomer carboxylic acids (FTUCAs), fluorotelomer carboxylic acids (FTCAs) and the x:3-acids could be determined, particularly in the aqueous phase of the industrial WWTPs. The further biotransformation of these intermediates led to an increased concentration of perfluoroalkyl carboxylic acids (PFCAs) in the effluent of WWTPs. Based on the frequency of detection and concentration of FTOHs, biotransformation intermediates (e.g. FTUCAs and FTCAs) and persistent biotransformation products, fluorotelomer-based precursors were identified as the most relevant precursors of PFCAs. Data for several corresponding WWTP influent, air and effluent samples suggests that FTOHs could be present as a residual synthetic intermediate of non-targeted PFASs, such as fluorinated polymers or other unknown low molecular weight fluorotelomer-based chemicals, which might be a further reason for the high frequency of detection. This should be investigated in future studies.

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Perfluorinated alkyl substances (PFASs) in household dust in Central Europe and North America

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In everyday life we come into contact with various chemical substances contained in consumer products. Perfluorinated alkyl compounds (PFASs) are one of the largest groups of such chemicals. PFASs have been used in a variety of applications such as surfactants, lubricants, in paper and textile coatings, polishes, food packaging, and fire-fighting foams for more than 60 years. They are added to consumer products to make them resistant to water, oil, stains and even fire. They are persistent, bioaccumulative and they have become ubiquitous. Several exposure pathways for PFASs have been identified: food and drinking water are the most important ones, followed by dust ingestion and indoor air. Humans spend almost 90% of their time indoors and moreover children mostly play on the floor, and hand-to-mouth activity is more significant than for adults. This can lead to higher exposure to PFASs than for adults. This work is part of a larger study within the Czech-American Scientific Cooperation Program aimed at identifying levels of contamination in residential indoor environments from three countries (Czech Republic, Canada and USA). The aim of the present study was to investigate and compare indoor concentrations of twenty PFASs in house dust samples collected in living rooms and bedrooms in 2013 from more than 30 homes of volunteers in these three countries. We also looked at differences between countries and compound patterns. Several PFASs were found in dust samples from more than 50 homes from three countries. Concentrations are of the same order of magnitude as in previous studies. Slightly significant differences were observed between samples from North America and Czech Republic. The dominant contaminant in all countries was PFOS but eight out of 20 target PFASs were detected in more than 50 percent of the samples. Among the carboxylic acids, PFHxA is a major contaminant in Czech samples while in North America PFOA showed the highest concentration. Spearman rank correlation gave good correlations between PFOA and shorter-chain carboxylic acids, suggesting that short-chain PFCAs are in use but still do not replace PFOA.

Interpreting Biological Effects of Metals and Their Mixtures

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Metals in the aquatic environment - interactions and implications for the speciation and bioavailability

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In most case scenarios, individual metals exist as components in mixtures with organic and inorganic substances and/or particulate matter. While the concepts encompassing mixture toxicity and modeling have been around for decades, only recently have new approaches (dynamic speciation techniques and fate and bioavailability models) been expanded to consider metal mixture scenarios. For example, the kinetic features of humic substances and inorganic colloids on the complexation of metals are generally considered. Although current environmental regulations rarely require an assessment of chemicals mixtures, research on these mixtures in the environment is essential for future regulatory demands and is vital for ensuring adequate environmental protection. Interpretation of speciation and bioavailability data from metal mixtures can be very complex and demanding, due to the existence of kinetic physicochemical transformations of the dynamic components. This kinetic effect largely affects metals' dynamic speciation, culminating in different transformed metal-containing products with different contributions for the metal uptake by a consuming interface. The environmental fate of metal mixtures, which determines how the mixture is biogeochemically processed and which receptors are most exposed (organisms and exposure route), with a special focus on their dynamic speciation, including a critical evaluation of the current challenges and available dynamic speciation techniques as well as computer codes and models, will be discussed.

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Chronic metal mixture toxicity to Ceriodaphnia dubia can be predicted using an independent action based bioavailability model

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Recently, several bioavailability-based models have been shown to predict acute metal mixture toxicity with reasonable accuracy. However, the application of such models to chronic mixture toxicity is less well established. In this study, we developed a chronic metal mixture bioavailability model (MMBM) by combining the existing chronic daphnidbioavailability models for the individual metals with the independent action model. The MMBM assumed that each metals binds to its own biotic ligand (BL) site and that metal (mixture) toxicity is related to the concentration of free metal ion binding to its metal-specific BL. Additionally, based on previous toxicity testing with Ni, Zn, and Pb mixtures, we assumed that Ni^{2+}, Zn^{2+} and Pb^{2+} do not compete for binding at the biotic ligand sites. To evaluate the predictive capacity of the MMBM, we investigated the chronic toxicity of a Ni-Zn-Pb mixture to *Ceriodaphnia dubia* in 6 (modified) natural waters differing in pH, Ca, and/or dissolved organic carbon (DOC) (pH range: 7-8; Ca range: 1-2 mM; DOC range: 5-12 mg/L). The toxicity of the Ni-Zn-Pb mixture was assessed concurrently with that of the individual metals. The predictive capacities of the chronic MMBM for mixtures was evaluated relative to that of the MMBM for the individual metals. Dissolved metal toxicity in the individual metal treatments, expressed as 7d-EC50, varied 4.3-fold, 3.3-fold, and 2.7-fold for Zn, Ni, and Pb depending on the water chemistry, respectively. The MMBM predicted the relative reproduction of 85% of the mixture treatments within 20% error, while the relative reproduction in the individual Zn, Ni, and Pb exposures were predicted within 20% error for 73, 83, and 70, respectively. This indicates that the MMBM predicted chronic toxicity of the ternary Ni-Zn-Pb mixture at least equally accurate as the observed in the individual metal treatments. Based on our study chronic MMBMs are a promising tool to account for the effects of water chemistry on metal mixtures during chronic exposure and could be used in metal risk assessment frameworks.

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Uptake and toxicity of copper and cadmium in single and mixtures exposure in the zebrafish, Danio rerio

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The aquatic environment receives a wide range of pollutants from a number of sources and is highly impacted by pollution. Due to the simultaneous presence of numerous pollutants it is important to investigate the effects of co-contamination. It has been shown that the presence of contaminants in mixtures can lead to a change in the effects of the individual contaminants resulting in synergistic or antagonistic toxicological effects on organisms. In this study we have investigated the uptake and toxicity (mortality) of copper (Cu) and cadmium (Cd) in adult zebrafish (*Danio rerio*) on the basis of 10-day and 28-day exposure experiments under different water hardness regimes. The experiments were conducted according to a partial factorial design covering a wide range of exposure concentrations. Survival and whole body metal and major cation concentrations were measured in all fish to determine whether Cu and Cd accumulation showed interactive effects and metal body burdens were predictive of toxicity. The levels of major cations were measured to determine whether loss of ion regulatory capacity was predictive of toxicity in the different single and mixture exposure scenarios. In the single metal exposures the zebrafish were generally much more sensitive to Cu than to Cd and the toxic effects showed a strong dependence on water chemistry. Exposure of the fish to binary mixtures of the metals resulted in a very strong synergisms when compared to the mixture toxicity predictions computed with the concentration addition (CA) model using the 10-day EC_{10} values for each of the metals. Toxicity also strongly increased with decreasing water hardness in the single exposures and the interactive effect between the two metals was observed at each of the water hardnesses tested. Neither the body burden nor the uptake rate of individual and mixture exposures explain the mortality observed in the different scenarios. An analysis of the major cation profiles showed that a significant loss of ions, in particular Na, has a large impact on the observed mortality.

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Departures from additivity in a wastewater sample spiked with binary mixtures of heavy metals

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Due to the well-known toxicity of heavy metals, monitoring and regulation of their concentration in the environment has been a very important issue in the past years. There are many methods for heavy metals detection based on physical and chemical techniques, however, such methods are not able to distinguish between available and non-available fractions of metals existing in the environment. In contrast to chemical methods, whole-cell bioreporters measure the bioavailable fraction of these analytes. Recently, we have mathematically developed a novel additivity framework for mixture research in the context of whole cell inducible biosensors [1].This new method is based on Concentration Addition (CA), also called Loewe Additivity. Our new approach proposes a multivariate extension of the effective dose (EDp) to take into account the occurrence of differential maximal effects presented by the mixture components, and also, an extension of Loewe Additivity that enables its direct application in a biphasic dose-response framework. Moreover, this method proposes a two-dimensional formulation of Loewe additivity computed for the two components of EDp, dose (*D*) and effect (*E*). The analyses of departures from additivity are based on Combination Index (CI) [2] for the *D* and *E* dimensions, where, $CI_{D\ or}\ CI_{E}D$ orCI_{E}=1 indicates additivity effect and CI_D or $CI_E > 1$ indicates antagonism. For global assessment of departures from additivity, a simplification of the two-dimensional information can be obtained by calculating a weighted index CI_w for any fractional effect *p*: $CI_w = CI_D \cdot CI_E$, where $CI_w > 1$ indicates overall antagonism, $CI_w = 1$ an overall additive effect, and CI_w [1] Martin K *et al*. (2015) *Scientific Reports* 5:17200 [2] Rodea I *et al*. (2010) *Water Research* 44:427:438 [3] Martin K *et al*. (2015) *Frontiers in Microbiology* 6:186 *Acknowledgement* – This research was supported by MINECO grant CTM2013-45775-C2-2-R

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Effect of temperature on Ni bioaccumulation and chronic toxicity to Daphnia magna

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The mechanisms involved in nickel toxicity are not fully understood. No information is available about the effect of temperature on chronic nickel toxicity to *Daphnia*. Therefore, our first objective was to investigate the effect of temperature on chronic nickel (Ni) toxicity and if a different effect is observed among four *Daphnia magna* clones. The second objective was to understand how temperature affects Ni uptake and elimination in four *D. magna* clones. At 15, 20 and 25°C 21-day life table experiments with Ni were performed with four *D. magna* clones taken from the same natural population. A linear model was built to predict the Ni effect on the reproduction per individual female(R_0) as function of temperature and clone. The effect concentrations (21 d EC_x) and concentration response curves were calculated for R_0 of all clones (to mimicked the effects on a population). Effect concentrations of the individual clones were also calculated. Uptake experiment was performed with the four *D. magna* clones exposed to 70 μg Ni.L^{-1} at 15, 20 and 25°C. Organisms were exposed to the stable isotope ^{62}Ni during 48h. The linear model built indicate that the effect of nickel on reproduction per individual female (R_0) was significantly affected by temperature and that this effect depended on the clone. The 21 d EC_{50}s based on R_0 of all clones (to mimicked a population) tested at 15, 20 and 25°C were 59.2, 74.3 and 121.2 μg Ni.L^{-1}, respectively. This represents a two-fold variation on the 21 d EC_{50}s. Significant interactions were detected between temperature, nickel and clones. After 48h of exposure nickel concentrations in Daphnids were lower at 25°C than at 15 or 20°C. No significant correlation was observed between 21 d EC_{50} and nickel accumulation after 48h of exposure. This suggests that it is not only the internal body concentration determines' the effect of temperature on Ni toxicity but that effect of temperature on the internal sequestration or toxicodynamics processes may also play a role in nickel toxicity. The results of the present study are in contrast with previous acute studies which indicate that acute metal toxicity increases with increasing temperatures. Additionally, on average chronic nickel toxicity decreased at higher temperatures. The present study showed that chronic nickel toxicity was significantly affected by temperature and this effect was different among the four *D. magna* clones tested.

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Fishing for mixture effects: impacts of diclofenac and cadmium on oxidative stress in a threatened indigenous fish species

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Pharmaceuticals and trace metals are increasingly prevalent in the aquatic environment due to anthropogenic pressures such as population increases. Although present at low concentrations, environmental persistence and high bioactivity of these chemicals can result in toxicological impacts on non-target biota. There is however, very little understanding of how emerging contaminants, such as pharmaceuticals interact with other toxicants. This study aimed to investigate the toxic impacts that occur when a native New Zealand fish, inanga (*Galaxias maculatus*), is exposed to mixtures of an emerging pharmaceutical contaminant, diclofenac (DCF), and a trace metal, cadmium (Cd). Inanga inhabits near-coastal freshwaters, and migrate through estuaries. As result they are potentially exposed to water bodies where toxicants are most concentrated. In laboratory studies, inanga were exposed to DCF (1000 µg L^{-1}), Cd (10 µg L^{-1}) and their mixtures (DCF, 1000 µg L^{-1}; Cd, 10 µg L^{-1}) for 96 h. The sublethal effects on hepatic oxidative stress were examined by measuring oxidative damage (lipid peroxidation) and antioxidant defence (catalase). There was a significant increase in hepatic catalase activity in fish exposed to Cd (10 µg L^{-1}) alone. However the increase in this important anti-oxidant defence enzyme was not sufficient to prevent an increase in lipid peroxidation, which also increased significantly relative to the unexposed control. Fish exposed to the Cd (10 µg L^{-1}) and DCF (1000 µg L^{-1}) mixture exhibited a different response whereby lipid peroxidation decreased compared to the individual Cd exposure. These results indicate that DCF is protective against metal-induced oxidative damage. These are the first such data to investigate the toxic effects of DCF and metal mixtures on fish. Understanding the impacts of toxicant mixtures, and the mechanisms by which they modify toxicity, is a critical need for the development of realistic environmental regulations.

Interactive effects of climate change and contaminants: environmental risks and human health implications

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The impacts of pharmaceutical drugs under ocean acidification: new data on single and combined chronic effects of Carbamazepine on Scrobicularia plana

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Ocean acidification (due to increased oceanic CO_2 uptake from atmosphere) and increasing discharges of pharmaceutical drugs, such as carbamazepine (CBZ), an antiepileptic, into the aquatic environment can exert toxic effects on marine organisms, including bivalves. However, the impacts of pH and CBZ when combined are poorly studied. Thus, the present study evaluated the chronic effects (28 days) of an environmental concentration of CBZ (3 μg/L) and the decrease of pH (to 7.1) acting alone and in combination on the marine clam *Scrobicularia plana*. CBZ quantification based on ELISA, protein content (PROT), electron transport activity (ET), lipid peroxidation (LPO) levels, glutathione S-transferase (GSTs), superoxide dismutase (SOD) and catalase (CAT) activities were determined. The mortality results indicated that a 28 days exposure to low pH (7.1) led to lower mortality (22%) than the exposure to single CBZ or the combination of CBZ and pH (33% for each) and that in individuals exposed to low pH mortality was recorded up to the first 96 h of exposure. This indicates an adaptive response of clams to the low pH (7.1), possibly due to changes in acid-base homeostasis, through an increase in the H^+ concentration. The exposure to CBZ led to an increase in Na^+ concentration, affecting the cellular acid-base homeostasis and resulting in higher mortality in clams exposed to CBZ and CBZ + pH. PROT increased after the exposure to CBZ and pH, single or combined, possibly indicating a higher rate of enzyme synthesis to prevent oxidative damage. The increase of GSTs activity under all exposure concentrations may be a consequence of that. ET decreased after all exposure conditions, revealing a lower metabolic rate as a defense mechanism to prevent the damage induced by these stressors. LPO results indicated higher levels in clams exposed to CBZ and pH, especially when combined. The activities of SOD and CAT were maintained or significantly decreased in comparison with the control, respectively, justifying higher LPO levels observed after exposure to CBZ, pH 7.1 and their combination. In conclusion, *S. plana* avoided oxidative damage under low pH for a long period (28 days), showing higher survival when exposed to this stressor compared to CBZ or CBZ combined with pH 7.1. The toxicity was higher when clams were simultaneously exposed to both stressors (CBZ + pH 7.1) since individuals' survival was reduced and oxidative stress increased when compared to single exposures.

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Coping with pharmaceuticals and personal care products (PPCP) in a changing ocean: bioaccumulation and ecophysiological implications in seabass (Dicentrarchus labrax) exposed to diclofenac

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Anthropogenic activities have led to great environmental challenges: remarkable chemical contamination and dramatic climate change. Both factors strongly affect marine ecosystems and are expected to worsen in the future, threatening marine species' welfare and survival. It is unknown how marine organisms will cope with the exposure to chemical contaminants under climate change. Given the lack of data and regulation for the the presence of the emerging the pharmaceutical and personal care products (PPCP), monitoring of these compounds in the aquatic environment becomes crucial. In this way, assessing climate change effects is even more imperative. Hence, the present work aimed to assess the synergistic effects between climate change and the exposure to the widely and massively used PPCP diclofenac, by analysing its bioaccumulation and elimination process in the European seabass (*Dicentrarchus labrax*), as well as the physiological and biochemical implications to this fish species. Results suggested that temperature and pH strongly influences the bioaccumulation and elimination of diclofenac, particularly it's partitioning in the different fish organs. Widespread impairments in fish enzymatic machinery, and a positive correlation between diclofenac accumulation and fish biochemical responses were observed. Hence, the deleterious synergistic effects of ocean warming and acidification combined with diclofenac exposure observed in the present work evidenced great biological challenges to marine vertebrate populations in the NE Atlantic coastal ecosystems in the future.

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Global warming causes conflicting effects on pesticide sensitivity: integrating multiple effects across latitudes
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Freshwater biodiversity is particularly vulnerable to global warming and pesticides. Both stressors are directly linked because most pesticides are assumed to become more toxic at high temperatures. This may, however, not reflect the net effect of pesticides in nature when their degradation is more rapidly at higher temperatures. Understanding how both stressors interact is therefore crucial to correctly estimate the ecological impact of pesticides in a warming world. Therefore, we investigated in larvae of the damselfly *Ischnura elegans* multiple effects of the pesticide chlorpyrifos (CPF) under a simulated global warming scenario on life history, thermal tolerance and physiology. By studying both low- and high-latitude populations we applied a space-for-time substitution to assess the role of thermal adaptation in shaping the vulnerability to CPF under global warming. CPF exposure negatively affected survival, growth rate, thermal tolerance, fat content and AchE activity and increased lactate dehydrogenase (LDH) activity. Under simulated warming CPF caused lower mortality, a less strong growth reduction (in high-latitude larvae) and less oxidative damage than at 20°C. This could be explained by the lower degradation of CPF at 20°C leading to a higher accumulation after four pulses compared to the treatment at 24°C. This indicates that testing effects of global warming on pesticide sensitivity may be misleading in experiments that keep pesticide concentrations artificially constant. Furthermore, this temperature effect on mortality was fine-tuned by local thermal adaptation with at a given temperature mortality being lowest in larvae tested at their local mean summer water temperature. This pattern suggests that the lower impact of a temperature increase of 4°C on the toxicity of CPF can be further strengthened if gradual thermal adaptation takes place in the high-latitude larvae. Notably, CPF exposure reduced the thermal tolerance and therefore the ability to deal with heat waves. This generates the complex scenario where exposure to CPF under a realistic field application scenario may cause lower direct negative effects under global warming, yet may cause higher negative effects in case of heat waves which are expected to increase in strength, duration and frequency under global warming.

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Exposure to a heat wave under food limitation makes an agricultural insecticide lethal
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Synergistic combinations of anthropogenic stressors are of major concern for biodiversity loss. While extreme temperatures and exposure to agricultural pesticides are becoming more frequent and intense under global change, their joint effects have been poorly studied. The potential for delayed interactions, and their modulation by the often co-occurring food limitation may be especially problematic, yet have been ignored. We tested for the effects of a transient heat wave combined with food limitation, and subsequent exposure to a widespread agricultural pesticide (chlorpyrifos) in *Coenagrion puella* damselfly larvae. The direct effects of the heat wave included a 3% increase in mortality and reductions in immune function (measured as activity of phenoloxidase, PO) and in metabolic rate (measured as activity of the electron transport system, ETS), which were not magnified by starvation. Starvation had both direct and delayed negative effects on growth rate, Hsp70 levels, total fat content, and activity levels of PO and ETS. Exposure to chlorpyrifos negatively affected all response variables, yet for mortality and immune function this was only in larvae previously exposed to the transient heat wave and starvation. This delayed synergism was especially striking for mortality: while the heat wave caused only minor mortality and starvation had no direct effect on mortality, chlorpyrifos caused considerable (48%) mortality, yet only in larvae that were previously exposed to both the heat wave and starvation. This could be partly explained by the inhibition of acetylcholinesterase (AChE) and the cumulative metabolic depression caused by each of these stressors. Furthermore, the delayed negative effects of the transient heat wave and starvation, combined with the direct negative effect of chlorpyrifos, considerably reduced larval growth rate. The here identified delayed synergism provides a novel explanation for the poorly understood potential of heat waves and of sublethal pesticide concentrations to cause mass mortality.

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Multi-biomarker responses between two dreissenid species in a global change context
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Under actual climate warming, further stressors such as contaminants might impact species distribution and ecosystems functioning. The ability of ectotherms to handle multiple stresses (*e.g.* a combination of metallic contamination and temperature change) will depend on their acclimation or adaptation capacities. Current literature raises the importance of understanding how organisms will respond and adapt to these changes in abiotic factors. Biomarkers can be used as very informative tools to evaluate exposure and effects of stressors on organisms. The freshwater bivalve *Dreissena polymorpha* is currently used in biomonitoring because it is a widespread species, tolerant to a wide range of environmental conditions and contaminants. It presents a sedentary lifestyle and is a filter feeder, often used to evaluate the accumulation of contaminants in its tissues and their subsequent effects on biological processes. However, *D. polymorpha* populations are declining because of their replacement by a sister species, the quagga mussel, *Dreissena rostriformis bugensis*. In this global change context, we wondered whether the quagga mussel might be more tolerant to environmental stressors, explaining its greater invasiveness. To evaluate the response pattern of both species, we exposed them to heat stress and metallic contamination. Mussels were placed in experimental beakers under five different treatments: control (no exposure), nickel (Ni) at 20 and 500 $\mu g\ L^{-1}$ and chromium (Cr) at 5 and 15 $\mu g\ L^{-1}$. Beakers were maintained at two temperatures: 12 °C, corresponding to the acclimation temperature, and 17 °C, to simulate a thermal stress. A battery of biomarkers, representative of both physiologic and antitoxic functions, was monitored (*e.g.* filtration activity, energetic reserves, antioxidant defences, detoxification mechanism). This multi-biomarker approach evidenced that the two species do not respond to multiple stresses the same way. We first showed that temperature do not affect biomarker responses four days after heat stress. However, we evidenced great differences between the two species, since biomarker responses were generally higher for *D. polymorpha* than for *D. bugensis*, particularly in non-exposed organisms. Even if the two species assimilated both metals the same way, the effects of contamination on biomarker responses were more marked in *D. polymorpha*, especially under nickel exposure.

Higher tier tests in the risk assessment of plant protection products (I)

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Aquatic mesocosm studies - use in risk assessment
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Mesocosm studies are a useful tool in aquatic risk risk assessment, as the highest experimentaltier in aquatic effects assessment. These studies are the only experimetal studies looking at porpulations and communities potential effects and recovery and as such aligned with the specific protection goals for aquatic invertebrates and primary producers. Furthermore they are the refence tiers against which lower tier approaches can be calibrated. Despite this, the conduct of such studies is reducing because they are becoming less accepted in regulatory risk assesment, as the interpretation of such studies and the assessments become ever more precautionary. The background to mesocosm studies and information on their design, conduct and interpretation can be found in the EFSA Aquatic Guidance. New validity criteria have recently been developed with respect to the number of sensitive and vulnerable taxa which should be present in the test systems and meet statistical criteria (appropriate minimal detectable difference – MDD) with the aim of ensuring that the effects could be detected and recovery observed. Strict adherence to these criteria can lead to studies being declared invalid and consequently ignored in the risk assessment. Despite not meeting the new criteria studies can still contain very useful information and should not be ignored in the assessment. Mesocosm studies can be bespoke to answer specific questions pose by the problem formulation and they should be evaluated similarly. Illustrated by examples, it will be shown how mesocosm studies can contribute to and inform the risk assessment, whilst potentially not fulfilling all validity criteria.

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How to deal with multiple micro-/mesocosms studies for the ETO and ERO-RAC derivation
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In the tiered effect assessment scheme developed for the EFSA Aquatic Guidance Document (2013), in principle, all tiers are able to address the ETO (Ecological Threshold Option). However, only the model ecosystem approach (micro-/mesocosm experiments) allows to address the ERO (Ecological Recovery Option) when addressing risk to algae, vascular plants or invertebrates. In order to address the reliability of the micro-/mesocosm experiments for the ETO and ERO-RAC derivation the AGD establish some requirements to be fulfilled (e.g. at least 8 potentially sensitive populations present in sufficient number with an acceptable MDD value). In this study we selected an insecticide as a case study

for which more than 6 micro-/mesocosm studies performed in different parts of the world and/or under different experimental condition to to assess if the power of the statistical test of these studies was sufficient to demonstrate treatment-related responses. From the 6 studies evaluated 4 of them comply with the criteria (at least 8 taxa of potentially sensitive taxonomic groups with MDDabu values < 100%) proposed by Brock et al. (2015). Two studies don't fulfil the criteria proposed as there were less than 8 potentially sensitive taxonomic groups belonging to Category 1 taxa. This can be partially explained by the fact that those studies were focused only on zooplankton and the number of taxa evaluated was lower than in the other studies. The results obtained after the re-evaluation of the data are similar to those reported previously in published reviews and scientific papers and in the new EFSA Aquatic Guidance Document with an effect class 2 concentration of 0.1 μg/L. This threshold level has been derived irrespectively of climatic test conditions (temperate, Mediterranean and also tropical as is the case of Thailand) and also even irrespectively of the exposure pattern (single application simulating spray drift and repeated application simulating run-off events). This can be explained by the fact that the most sensitive taxa (crustaceans and insects) were well represented in the cosms evaluated.

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Stream mesocosms in the context of insecticide risk assessment
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In the risk assessment of plant protection products (PPP) aquatic mesocosm experiments are used as higher tier tools to analyse effects in an ecosystem context under more realistic conditions than in single species tests. Therefore, mesocosm experiments should simulate realistic exposure scenarios and should feature a representative biocenosis including potentially sensitive species. Usually these experiments are conducted in lentic mesocosms (artificial ponds or enclosures) for regulatory purposes. But in certain cases lotic mesocosms (artifcial streams) may be the more appropriate choice to describe the ecotoxicological risk of a PPP in the aquatic environment. Many different factors have to be considered to decide between lentic or lotic mesocosms: The environmental behaviour of the test substance, exposition scenarios, intrinsic sensitivity of freshwater species and their vulnerability derived from their ecology. In this presentation we want to discuss these factors and bring some light in the discussion by presenting results of an experiment with four stream mesocosms in comparison with simultaneously performed pond mesocosm studies. In addition we compare the artificial stream data with the data of two local natural streams representative for a landscape with moderate agricultural use. Finally we present the results of a pilot stream mesocosm study with the insecticide carbaryl. The comparison study shows that the macroinvertebrate community of the stream mesocosms corresponds well with the reference streams regarding diversity, feeding types, ecological traits and number and abundance of taxa, which are potentially sensitive and vulnerable. Abundance and number of taxa with a high intrinsic sensitivity against carbamates, organophosphates and neonicotinoids was higher in the artificial streams than in the pond mesocosms. On the other hand, for pyrethroids the lentic mesocosms seem to be more meaningful caused by the occurrence of the focal species *Chaoborus*. First results of the carbaryl study shows, that the most sensitive macroinvertebrate taxa are Plecoptera, Gammarids, the mayfly *Ephemera danica* and the caddisfly *Polycentropus flavomaculatus*, all taxa prefering or restricted to lotic water bodies.

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The Landau stream mesocosm facility
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In the current tier-3 risk assessment for plant protection products (PPPs) in edge-of-filed surface waters risks are mainly based on pond-like mesocosm approaches. However, transient and dynamic PPP exposure scenarios as observed in lotic systems are hardly achievable in pond-like mesocosm approaches. The present compilation of studies performed at the Landau stream mesocosm facility provides knowledge on dynamic PPP exposure scenarios at different time scales (i.e. peak-, hour- and day-scale) under flow-through and recirculating conditions. To address the effects of a pesticides on aquatic plants under a day-scale exposure, the submerged macrophyte species *Elodea canadensis* and *Myriophyllum spicatum* were exposed to a sulfonylurea herbicide for 24 hours (in recirculating flow mode) followed by a subsequent PPP-free flushing period to enable a recovery period of 42 days. Two macrophyte species revealed pronounced adverse short-term effects on shoot growth and yield, but also a subsequent recovery at the end of the experiment. Preliminary to the effect assessment of insecticides on hour- or day-scale the setup of stream mesocosms with macrophytes and aquatic organisms was considered in general. Abundance and robustness of invertebrates was evaluated including sensitive and/or vulnerable species. Non-dosed streams were used to evaluate the influence of different aquatic macrophyte species and the duration of the pre-experimental period on the establishment success of macroinvertebrates. To address the stream-internal longitudinal changes of the concentrations and the residence time of PPPs a location-dependent invertebrate sampling scheme was used within the scope of a 6-hour application of the insecticide etofenprox (0.05, 0.5 and 5 μg/l; n=4). Short-term effects were observed on population and on community level. The sublethal endpoint drift was revealed as most sensitive as significant effects were visible occasionally at 0.05 μg/L for Simuliidae and at 0.5 μg/L for potentially sensitive species as Polycentropodidae. An integrated stream mesocosms test design capable of identifying inter-ecosystem boundary effects was established using the widely distributed web-building spider *Tetragnatha extensa* as a representative riparian species. On different scales of complexity, this approach may be used to evaluate PPP transfer due to emerging insects, bioaccumulation of PPPs in riparian spiders and alteration of trophic structures in riparian ecosystems.

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Effects of chronic exposure to thiamethoxam on a summer generation mayfly population in an outdoor mesocosm
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The effect of neonicotinoids on mayfly nymphs has been investigated in recent literature, demonstrating they are amongst the most sensitive aquatic insect taxa to these insecticides. A publication by van den Brink et al (2015) reported the chronic effects of a continuous thiamethoxam exposure (28d) under laboratory conditions on over-wintering generation mayfly larvae (*Cloeon dipterum*); 28d EC_{10} = 0.43 ug a.s./L. A GLP outdoor mesocosm study was performed to investigate chronic effects of a continuous exposure to thiamethoxam on a summer generation mayfly (*Cloeon dipterum*) population. Twenty stainless steel enclosures within one large pond enclosure were used for the study; five untreated controls and five treatment levels with three replicates each. The treatment levels were, 0.1, 0.3, 1.0, 3.0, 10 µg a.s./L. Concentrations of thiamethoxam were maintained with twice-weekly applications to the water column via mixing. The time weighted average concentrations ranged from 93 to 108% of nominal, with a mean of 101% of nominal. Mayfly abundance was assessed with sweep-netting and substrate sampling (two baskets with aquatic macrophytes and stones). Adult emergence was also sampled. Mayfly sampling occurred weekly for the duration of the study. The % minimum detectable difference (MDD) values for larval abundance ranged from 37 to 49% up until day 27 of the study, indicating that small effects could be detected. On day 34, the MDD value was 87%, due to the natural population decline. The no-observed-effect-concentration (NOEC) for larval abundance was determined to be 0.3 μg a.s./L and the lowest-observed-effect-concentration (LOEC) was determined to be 1.0 μg a.s./L.The results for adult emergence support the observed effects on the larval population. The results of the study indicate that under conditions of continuous exposure, there is no apparent difference in sensitivity to thiamethoxam between summer generation mayfly larvae tested under field conditions and over-wintering generation mayfly larvae that were tested under laboratory conditions. This study adds to the growing debate on the effects of neonicotinoids on sensitive aquatic insects by determining a no effect level for mayfly populations in outdoor mesocosms continuously exposed to thiamethoxam, representing worst-case conditions with respect to FOCUS modelling drainage scenarios.

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Experiences from a suite of higher-tier laboratory tests on aquatic larvae of four potentially-sensitive insects, and an amphipod, with the aim of comparing sensitivity to an insecticide
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The EFSA Guidance document on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters (2013) makes recommendations for assessing the risks to sensitive and vulnerable invertebrate taxa. Questions about such taxa can arise when the results from a static microcosm study are interpreted in a wider context. This is especially the case for taxa which are not represented within the microcosms, such as stonefly larvae which are found in *flowing* waters. Published comparative-assessments have suggested that invertebrates such as stonefly larvae (Plecoptera), commonly used as indicator species for water quality, are particularly sensitive to insecticides. Therefore, actually testing their *relative sensitivity* compared with taxa which are found in microcosm studies, could minimise uncertainty (and therefore the 'uncertainty factor') when implementing microcosm results in the risk assessment. A set of laboratory tests on an insecticide was undertaken by Cambridge Environmental Assessments (CEA) to assess relative sensitivity of larvae of Plecoptera (stonefly), Megaloptera (alderfly) Trichoptera (caddisfly), and to 'benchmark' this against tests on *Chaoborus* and *Crangonyx* – both of the latter two being present and sensitive to this insecticide in previous microcosm experiments. To achieve this, a novel laboratory test design using microcosm water and sediment was developed. Plecoptera and Megaloptera were collected from rivers in Southern England and Trichoptera, *Chaoborus* and *Crangonyx* were sourced from CEA microcosms.

Separate tests were conducted for each taxon using 3-litre beakers containing sediment, microcosm water, alder leaves as a source of detritus, with gentle aeration, and pebbles to simulate the natural environment of stonefly larvae. After addition of the insecticide, observations were made during 96 hours, and final counts of survival were recorded. The results were used to determine the relative sensitivity. Practical experiences will be shared on selecting test organisms based on life history and abundance, and conducting and assessing the sensitivity of these non-standard species in laboratory.

Development, standardization and implementation of LCA and integration with economics for transportation infrastructure and operations

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A multi-objective optimization-based pavement management decision-support system for enhancing pavement sustainability

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In a society where the public awareness of environmental protection is increasing remarkably and the availability of resources and funding is limited, it is more vital than ever that departments of transportation (DOTs) and decision-makers (DMs) seek new tools that enable them to make the best and most rational use of these resources, taking into account environmental and social factors, along with economic and technical considerations. However, the practice adopted by highway agencies with regards to pavement management, has mostly consisted of employing life cycle costs analysis (LCCA) systems to evaluate the overall long-term economic efficiency of competing pavement design and maintenance and rehabilitation (M&R) activities alternatives. This way of supporting the decision-making process as it relates to pavement management, in which little or no importance is given to environmental considerations, does not seem to be effective in advancing sustainability in pavement systems. In view of this, it is clear there is an urgent need for pavement management decision-support systems (DSS), which, by integrating multi-disciplinary and complementary pavement life cycle modelling approaches, enable the DMs to properly account for, consider and assess the cumulative and long-term impacts of their decisions and practices regarding sustainability goals and targets. This only can be achieved by employing techniques and tools provided with a comprehensive and wide-scoped cradle-to-grave capacity of analysis. To address this multifaceted problem, this paper presents a comprehensive and modular multi-objective optimization (MOO)-based pavement management DSS which comprises three main components: (1) a MOO module; (2) a comprehensive and integrated pavement life cycle costs - life cycle assessment (LCC-LCA) module that covers the whole life cycle of the pavement; and (3) a decision-support module. The potential of the proposed DSS is illustrated with a case study consisting of determining the optimal M&R strategy for an one-way flexible pavement section of a typical Interstate highway in Virginia, USA, which yields the best tradeoff between the following three, often conflicting, objectives: (1) minimization of the present value (PV) of the total life cycle highway agency costs (LCHAC); (2) minimization of the PV of the life cycle road user costs (LCRUC); and (3) minimization of the life cycle greenhouse gas emissions.

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Aggregation Errors in Life-Cycle Assessment of Freight Trucks

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Life-cycle emission factors for heavy-duty trucks found in the literature and life-cycle assessment (LCA) databases typically reflect a generic or industry-average perspective in global or regional context. As a result, emission factors often lack the specificity to accurately characterize the performance of the vehicles, which could lead to misdirected strategies and actions. We believe that by collapsing over many individual vehicles to establish a formal emission factor, this aggregation process restricts the explanatory power of LCA for individual cases by removing within-group information that may be useful for policy-making. This information includes knowledge of specific trip attributes (location, road type, time of day, etc.), vehicle attributes (gross weight vehicle rating and payload), driving conditions (levels of congestion), and characteristics of their respective supporting infrastructure systems (location of critical infrastructure and route topology). We assess the accuracy of greenhouse gas (GHG) emission factors of heavy-duty trucks by accounting for how they are utilized in real life and the sensitivity of GHG emission factors to vehicle loading factors (payloads) and commodity types transported. Case studies suggest that emission factors based on average payload always underestimate emissions. We provide examples of how existing emissions inventories change in response to infrastructure, vehicle, and payload characteristics. This research advances the scientific community's understanding of how GHG emissions from heavy-duty trucks can be allocated on more resolved spatial and temporal scales, thereby improving decision-making on a case-by-case basis.

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Prospective Fleet LCA of Swiss Air Transport

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This work presents a Life Cycle Assessment (LCA) of passenger aircraft travel at both the individual aircraft and Swiss fleet level with projections until 2050. In the first stage of this work we perform LCA of three different classes of aircraft with construction years ranging from 1970 to 2050. Parameters such as aircraft material composition, weight, lifetime distance travelled, fuel consumption, operating emissions, and seat load factor are modelled as variables dependant on the size of the plane, construction year, and flight distance. Future fuel consumption and operating emissions are modelled for two scenarios reflecting either a continuation of historic improvements (BAU), or an optimistic acceleration of technology improvements (OPT). Future Swiss air transport demand is modelled to increase significantly at 2.75% per year. Flight length distributions, Fleet composition, and aircraft use parameters are based on Swiss and European statistical data. Results are presented for ReCiPe midpoint for indicators: climate change, terrestrial acidification, photochemical oxidant formation, and particulate matter formation. Results show that environmental impacts are strongly related to fuel consumption. For climate change and acidification this is due to combustion in the cruise phase, while the fuel production phase is most important for other indicators, though impacts from landing and take-off cycles were also significant. Results for all impact categories varied by more than 50% depending on aircraft size and construction year. Flight distance is found to have a strong impact on per passenger kilometer (pkm) environmental impacts for flights below 1000 km, which make up approximately 20% of all pkm. Fleet average environmental impacts per pkm have decreased by over 40% since 1990, and are found to decrease by another 40 to 60% by 2050 for BAU and OPT technology development scenarios, respectively. The total pkm performance of the fleet is forecast to increase by over 2.5 times by 2050 compared to 2015. These large increases in future demand are expected to leave Swiss fleet level environmental impacts at levels similar to today's, even with greatly accelerated technology development. Thus, reductions in fleet impacts from Swiss passenger air travel are only likely in the future with growth rates much slower than those currently predicted. This work was completed within the SCCER Mobility (www.sccer-mobility.ch).

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Using life-cycle assessment to guide the development of bio-based fuels, lubricants, rubber, and plastics

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Decarbonizing the transportation sector is critical to achieving global climate change mitigation. Although biofuels will play an important role in conventional gasoline and diesel applications, reducing societal reliance on oil and gas cannot be eliminated unless the full suite of products can be replaced. In this study, we explore the climate implications of producing bio-based fuels, lubricants, and precursors for rubber and plastics. We show that, guided by life cycle assessment combined with linear programming, integrated sugarcane biorefineries for producing jet fuels and lubricants could be built to minimize the overall GHG impact or maximize total energy output through novel combinations of furan and fermentation pathways.

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Using LCA for Comprehensive Sustainability Assessments of Road Pavements

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A review of sustainability assessment tools for transportation systems shows that sustainability is often evaluated based on the goal of improving the environmental state (Brodie et al. 2013); however, comprehensive sustainability must also account for improvements to the social and economic state of society. In pavement rating tools applied at the project level, such as BE^{2}ST-in-Highways and GreenPave, environmental impacts are evaluated using LCA, but tools are needed in addition to LCA to support decision making for comprehensive sustainable outcomes. The LCA methodology has been expanded to apply to economic aspects through LCCA and attempts have been made to measure social aspects similarly (SLCA). This paper reviews several LCA methods as environmental calculators for use in sustainability assessments of road pavements and explores LCCA and SLCA methodologies. The results highlight the uses and limitations of LCA, LCCA, and SLCA for comprehensive sustainability evaluation and provide insight into approaches to address limitations and to improve sustainable decision making at the project level.

Biological effects of emerging micro pollutants at realistic environmental concentrations (II)

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Effect of environmentally realistic doses of pesticides on tritrophic interactions in grass strips
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In agricultural areas, grass strips implemented as buffer zones to protect water quality are regularly subjected to residual pesticide contaminations mainly resulting from runoff. Grass strips are also reservoirs of biodiversity for plants and insects that are thus potentially exposed to these compounds. Within plant-insect trophic chains, interactions between trophic levels are governed by exchanges of resources (nutrients, water) as well as chemical signals. As a consequence, each trophic level can regulate lower (top-down control) and higher (bottom-up control) trophic levels. This work aimed at studying the effects of environmentally realistic low doses of pesticides on the interactions between *Lolium perenne* (ryegrass), an aphid pest *Rhopalosiphum padi* and its parasitoid *Ephedrus plagiator*. To simulate a runoff-resulting pesticide exposure of the trophic chain in a context of grass strip, the plants were exposed *via* their root system to an herbicide (isoproturon) and an insecticide (Thiamethoxam), applied alone or in combination. Insect exposure was thus related to pesticide transfer along the trophic chain. We found that the three trophic levels were affected by pesticides, the combination of both compounds resulting in synergistic, additive or antagonistic effects depending on pesticide dose and traits measured. Moreover the observed effects were found not to be always linked to deterioration in the quality and / or quantity of resources produced by lower trophic levels, suggesting actions of pesticides on non-target organisms as chemical stressors and/or potentially alteration of plant-insect chemical signals. However, pesticides effects can be offset between biological organization levels. Thus, in agricultural landscapes, the interactions between plants, herbivores and their natural enemies are subject to change with pesticide exposure, even at low doses typically found in grass strips.

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Signalling and regulation pathways involved in cryptic effects induced by residual levels of pesticide degradation products on higher plants
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Pesticides are pollutants of high concern due to environmental ubiquity resulting from extensive use in modern agriculture and persistence in soil and water. Study of plant behaviour toward such molecules and their breakdown products in situation of residual pollution is important to evaluate toxicity at low concentration in the context of environmental risk assessment. Studies at various spatial scales have highlighted frequent occurrences of major herbicide breakdown products in surface and ground water. In order to understand mechanisms underlying the impact of such molecules, the model plant *Arabidopsis thaliana* was confronted with low levels of breakdown products of widely used herbicides (aminomethylphosphonic acid for glyphosate, desethylatrazine and hydroxyatrazine for atrazine), quantified in soils of field margins in an agriculturally-intensive area. Integrative analysis of physiological, biochemical and molecular responses showed that these residual xenobiotics rapidly led to cryptic effects with major reorientations of carbon and nitrogen metabolism and gene expression changes. The extent of such changes, in absence of significant action on primary targets, was consistent with involvement of early regulatory processes and signalling mechanisms. In order to identify such xenobiotic-related signalling, *Arabidopsis* seedlings were subjected to low levels of structurally-related xenobiotics (atrazine, hydroxyatrazine, desethylatrazine) under conditions of No Observable Adverse Effects, and a genome-wide transcriptomic analysis (CATMAv7 microarray) was carried out. Functional analysis of differentially expressed genes and of their promoters revealed that signalling pathways related to plant hormones (cytokinins, ABA), low energy sensing (MYB2), environmental stresses (light, drought, heat, oxidative stress, hypoxia) and biotic interactions (WRKY40) were involved in xenobiotic signal transduction. Further studies will use hormone analogues and inhibitors, or mutants related to hormone signalling, in order to analyse xenobiotic-hormone interactions. Characterisation of xenobiotic signal transduction pathways should be of interest for herbicide management in crop systems and for protection of wild plant communities and ecosystem functioning. Identification of crosstalk processes between xenobiotic, abiotic and biotic stress signalling may give novel insights into the interplay between chemical pollution and climate change stressors.

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The effect of marine litter at cell level: cytotoxicity evaluation of common nanomaterials and emerging contaminants
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During the past decade, new technologies were based on the use of efficient and cheap alternative materials, according to industrial interest. Therefore, a lot of common commercial products are currently made of nanomaterials or synthetic materials, such as plastic. Their input to the environment, years after years, contributes to increase the number of waste in coastal waters, estuaries and oceans. This is also known as marine litter. Therefore, the aim of recent several researches is to study the toxic effects of these compounds, the environmental deterioration and human health effects caused by them. The scope of this study was to understand the toxicity of common NMs and emerging contaminants at cell level in terms of oxidative stress and cell viability and to compare the cytotoxic response of two different cell lines exposed to the same conditions. In addition, with regards to carbon based nanomaterials, such as fullerenes (C_{60},C_{70},..), due to their sorptive properties, they may act as carriers for other contaminants, providing rapid and long-range transport or immobilizing them. The change in the bioavailability of other contaminants is known as Trojan Horse Effect. In the present work, the cytotoxicity of nanomaterials (including fullerene soot, graphene nanoflakes, and several other of metal and metal oxide nanoparticles) has been evaluated in vitro using two different cellular lines: T98g, cerebral human cells, and HeLa. The cells were exposed during 24-48 h to different level of contaminants from 10 ng/ml to 60 μg/ml. Furthermore, cells were also exposed to increasing concentration of different binary mixtures containing fullerene soot, in order to evaluate Trojan Horse Effect. The cell viability and cell's oxidative stress were measured via High Content Analysis. Cells were selected as objects in two channels with two different wavelength (λ_1=485 nm and λ_2=549 nm) close to the Hoechst and DHE emission maximum, respectively. The obtained results contribute to the current knowledge of the cytotoxicity of both nanomaterials and microplastics. According to them, oxidative stress is one of the mechanisms that explain the toxicity of these emerging contaminants at cell level, as has been observed for both cell lines. In addition, carbon nanomaterials have the potential to interact in a complex way with the co-occurring contaminants, changing the bioavailability and, therefore, their resulting toxicity in either a synergistic or an antagonistic manner.

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Seabream macrophages responses to combined stressors: gene expression changes
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Nanoparticles (NPs) and pharmaceuticals are among the different classes of contaminants of emerging concern (Buchberger 2007, Sauve and Desrosiers 2014). NPs are increasingly used in several areas of human activity, despite the lack of knowledge regarding their impact in ecosystems. Pharmaceuticals have a bioactive nature and their consumption is increasing worldwide. Further, their removal by wastewater treatments is not sufficiently efficient, and in some cases more toxic or slowly degradable transformation products are formed (Oliveira et al. 2014). This concern may be emphasized taking into account that, in the environment, such contaminants may occur simultaneously and their interaction may become more harmful. Few studies have focused on the combined effects of these contaminants on coastal and estuarine ecosystems. This study aimed to test the *in vitro* effects of gold nanoparticles (AuNPs), with different coatings, and the human pharmaceutical gemfibrozil (GEM) in gilthead seabream (*Sparus aurata*) macrophages. Macrophage gene expression after exposure to these contaminants (individual or combined exposures, and combined with bacterial lipopolysaccharide, LPS) was assessed focusing on different pathways (e.g. apoptosis, inflammatory responses, antibacterial responses, oxidative stress or calcium signalling). The present study revealed different patterns of response to AuNPs with different coatings. Citrate coated AuNPs induced expression of genes such as lysozyme, interleukin-1P (IL1P) or NAPH oxidase (NCF4). Polyvinylpyrrolidone coated AuNPs elicited downregulation patterns of genes such as caspase 1 (CASP1), tumour necrosis factor P (TNFA) or extracellular calcium sensing receptor precursor (GPCR). It is also noteworthy that, with the exception of IL1ю, all the other genes showed downregulation for the ternary mixture treatment AuNPs+LPS+GEM. In the combined exposure of AuNPs+GEM, genes related to similar pathways (CASP1 / TNFA or NCF4 / GPx4b) presented opposite patterns of expression. Gilthead seabream macrophages showed to be a useful in vitro model to investigate combined effects of these emerging contaminants.

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Biological-based profiling of wastewaters from sources to the discharge into the environment
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Aquatic ecosystems are widely contaminated by trace micropollutants that may represent a risk for human health and wildlife. Even if some compounds are well removed by wastewater treatments, the discharge of effluents remains one of the main entry of several micropollutants in the environment. It is particularly worrying for the compounds that are endocrine disruptors and/or that can induce genotoxicity. New approach using quantitative biological activities has to be applied along the water line, especially to monitor the pollution of wastewater. The objective of this study was to apply a panel of *in vitro* bioassays to determine water contamination profiles from the wastewater network to the outlet of the WWTP. A panel of bioassays were applied on various samples collected in six sites on the network, along the WWTP in order to evaluate the impact of biological treatment and tertiary ozonation. Selected i*n vitro* bioassays, applied on water SPE extract, enabled detection of active chemicals responsible of endocrine disruption (estrogen, androgen, progestogen, mineralocorticoid, glucocorticoid, pregnane X and dioxin receptors) and the genotoxicity was evaluated by using the SOS Chromotest. In all sites along the wastewater network, ER, AR, MR activities and genotoxicity were detected. GR activity was measured at only three points of the network and was particularly important in the WWTP influent. Endocrine disruptor activities as well as genotoxicity were detected in raw wastewater (WWTP influent). Biological treatment highly reduced ED activities and genotoxicity. Nevertheless residual activities were still detected in the tertiary effluent. Results of the 1st sampling campaign allowed the identification of three notable points of interest on the network and at the WWTP influent. Results of two next sampling campaigns will help to confirm these “hot-spot” sites in order to apply effect-directed analysis to perform chemical identification of the compounds responsible of endocrine disruption activities and/or genotoxicity.

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Effects of magnetic microparticles used for phosphorus removal in eutrophic waters on plankton community
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Eutrophication is a worldwide concern causing environmental degradation and compromising water resources use. Magnetic particles have been recently proposed as a promising method to adsorb and remove phosphate from lake water and sediment. However, before this method is applied into the whole-lake experiment, ecotoxicological studies must be perform to assess the effect of such microparticles on the aquatic community. In previous studies, EC50 for standard specie as D. magna and Chironomus sp. has been assessed, here we present a microcosms study to further explore the effects of these particles on the whole community. Our specific hypotheses were: a) the addition of MPs will cause transient effect on the structure of plankton community allowing its recovery by the end of the experiment and b) a top-MPs spike will prone more changes on plankton community than a bottom addition because of a longer exposition time.In order to test these hypotheses, a microcosms experiment with three different treatments of MPs (i.e., control, bottom_addition and top_addition) was set-up. Bottom addition simulates an addition of the particles directly into the sediment; and, top addition if it would be spiked at the surface of the water column. The amount of amended MPs was the same for bottom and top addition treatments and it was based on the calculations of how much MPs are necessary for trapping all sediment mobile P pool (P_{Mobile}). However, and based on previous studies, we decided to add the double of that value in order to counteract chemical interferences. This experiment has two goals: first, to assess the effects of magnetic particles on the plankton community; and, second, to estimate the efficiency of phosphorus removal by the mpFe.The experiment was performed in outdoor microcosms (40 L volume) consisting of a stabilization period of 10 days, particles addition and retrieval after 24 hours simulating real field application, 79-days assessment under aerobic conditions and 5-days (stating on day 80) under anoxic conditions. Plankton abundance, species composition and richness are to be determined. Neither ChlB*a*_or oxygen concentration denoted statistical differences along the experiment between control and treatments. It may mean no changes of phytoplankton abundance or zooplankton grazing pressure but may hide community structural and richness changes. Based on chemical analysis, phosphorus was successfully removed by the MPs added with no detectable effect on the current biological parameter assessed.

Ecotoxicology and risk assessment of nanomaterials - Interactions at nano-bio interface (III)

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Effects of engineered nanoparticle-contaminant mixture exposures to aquatic organisms
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Due to their high surface reactivity engineered nanomaterials (ENMs) can interact with other anthropogenic contaminants, potentially influencing their environmental distribution, bioavailability and toxicity. Suggested mechanisms behind those altered toxic effects include changes of contaminant bioavailability caused by contaminant adsorption to ENMs, or an altered toxicity due to interactive toxic effects of mixture exposures. Here we want to summarise results from a range of experiments investigating the effects of several ENM-contaminant mixtures: carbon nanotubes (CNTs)-phenanthrene; titanium nanoparticles (TiO_2NP)-benzo(a)pyrene; silver nanoparticles (AgNP)-water soluble faction of crude oil (WSF); and AgNP-17?-ethinylestradiol. Studies included different aquatic organisms, reaching from the unicellular freshwater algae *Pseudokirchneriella subkapitata* to the marine flatfish *Scophthalmus maximus*. The ENMs were characterised and their behaviour in the tested environmental matrices was determined. ENM-contaminant interactions, their uptake in organisms and effects on contaminant bioavailability were investigated. To determine potential changes in toxic effects, suitable endpoints or biomarkers were analysed for each test species. All tested nanomaterials were found to be taken up or to be associated to organisms' surfaces; however interaction between ENM and contaminants and effects on bioavailability and toxicity varied. Adsorption of hydrophobic PAHs was observed to CNT and TiO_2NP. Undergoing aggregation, both CNTs and TiO_2NPs reduced dissolved PAH concentrations. However, both dissolved and ENM-associated appeared to remain bioavailable to varying extends. Similarly, both, single and mixture exposures of AgNP and the WSF of North Sea crude oil, caused activation of oxidative stress related biomarkers in calanoid copepods. The presence of AgNP did not significantly change 17?-ethynylestradiol bioavailability to juvenile turbot. Impacts on steroid hormone concentrations in mixed exposures followed 17?-ethynylestradiol single contaminant effects, however, impacts on some hormones were slightly increased in mixed exposures. ENM-contaminant mixture effects do not follow a general mechanism. Their interaction and mixture toxicity depends on multiple factors, including ENM material and contaminant properties. Further research is needed in order to provide sound data for ENM environmental risk assessment, which needs to include risks from ENM-contaminant mixtures.

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Long term toxicity of Ag-NM on soil nitrifying bacteria after biotransformation in WWTPs
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Silver nanomaterials (Ag-NMs) are used in many cosmetics and textiles because of their antimicrobial properties. Several studies have shown that silver is released from textiles during washing and reach wastewater treatment plants (WWTPs), where significant amounts adsorb to sewage sludge. As sewage sludge is used as fertilizer in agriculture, adsorbed NMs enter the soil resulting in a negative impact on soil microorganisms. The aim of our study was to investigate the influence of sulfidation as one of the observed transformation processes occurring in WWTPs on the bioavailability of Ag-NMs. Ag_2S is less toxic than Ag^+, therefore, a comprehensive risk assessment regarding the pathway “influent – WWTP – soil” requires information on the effect of sulfate in wastewater on the bioavailability of Ag-NMs. For that reason we first simulated the transformation processes in laboratory WWTPs. In a second step the processes during sludge conditioning were simulated using two different methods. The sludges were split into half, one half was directly applied into soil the other half anaerobically digested before mixing the biosolids into soil. To investigate potential effects on soil microorganisms the ammonium oxidation test (ISO 15685) was performed at regular intervals. Sewage sludge and soil samples were investigated with TEM EDX. In the simulation of WWTPs no negative impact on the microbial activity was observable. The soil ammonium oxidysing bacteria in the directly applied treatments were more than 50 % inhibited, the anaerobically digested ones nearly complete. The different sulfate levels had no influence on the bioavaiability of Ag-NM. The TEM EDX analysis also showed no differnce between the to sulfate treatments. After processing The particles EDX spectra in the sewage sludge indicated Ag_2S by a perfect matching 2:1 ratio and the particle size was those of the pristine material NM-300K. The EDX spectra after long term incubation in soil samples indicated Ag_2S as well, but we also found different Ag-species. Although we found mostly Ag_2S in sewage sludge and soil the long term study with ammonium oxidising bacteria resulted in strong inhibition in all treatments. Therefore it can not be concluded that the treatment of wastewater and sewage sludge inevitably results in nontoxic Ag-transformation products.

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Toxicity of TiO2 nanoparticles on soil nitrification at environmentally relevant concentrations: Evidences for an absence of classical dose-response

relationships

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Nitrification is a crucial microbiological process for soil fertility depending on the combined activity of ammonia- and nitrite-oxidizers. This key step of the nitrogen cycle is known to be very sensitive to environmental stressors. However, the impact of emerging pollutants such as titanium-dioxide nanoparticles (TiO_2-NPs) which are increasingly released in agricultural soils, remains to be investigated, especially at very low concentrations (< 1 mg kg^{-1} dry soil) (Sun et al. 2014; Simonin & Richaume 2015). We assessed the impact of eight TiO_2-NPs concentrations ranged from 0.05 up to 500 mg kg^{-1} dry soil in soil microcosms on nitrification activity and abundance of ammonia-oxidizing archaea (AOA), bacteria (AOB) and nitrite-oxidizers (*Nitrobacter* and *Nitrospira*) using quantitative PCR. In addition, aggregation and oxidative potential of TiO_2-NPs were measured in soil solution to identify the main drivers of TiO_2-NPs toxicity in soil. After 90 days of exposure, AOA appeared to be the most affected by TiO_2-NPs, while *Nitrospira* seemed insensitive to this contamination. AOB and *Nitrobacter* abundance exhibited similar responses. The activity was reduced by the lowest (0.05 mg kg^{-1}) and the highest (100 and 500 mg kg^{-1}) TiO_2-NPs concentrations. Interestingly, all indicators exhibited a non classical dose-response relationship that did not enable to calculate effect concentrations. This results could be linked to the influence of NPs concentration on the aggregation and oxidative potential of TiO_2-NPs assessed in soil solution. In fact, TiO_2-NPs aggregation was increased with concentration, while oxidative potential was consequently decreased. Thereby, TiO_2-NPs exhibited the largest toxic potential in soil solution at the lowest concentrations that may explain the negative effects found at very low concentration (< 1 mg kg^{-1}) on nitrification activity and nitrifiers abundances. Altogether these results pointed out the need to include very low concentrations of NPs in soil risk assessment studies. This study also emphasized that classical approaches for risk assessment based on dose-response toxicological tests are not pertinent for NPs in soil and highlights the necessity to characterize NPs properties for each concentration used in conditions close to those encountered in soil.

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There are more than species differences: challenging the immune system with nanoparticle's sex-specific biological identity

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We have previously reported that, through formation of a protein corona, the same nanoparticles can acquire a quite different biological identity depending on the species-specificity of the proteome to which they are exposed, and that they likely differ in their fate under immune vigilance. Here we hypothesise that if the protein repertoire diversity matters in general (not simply because of significant cross-species variations), sex differences in the proteome would translate into differential corona formation even within the same species. In this study, we show for the first time that the protein repertoire differences by sex determine nanoparticle's sex-specific biological identity, and that immune cells in zebrafish interact differentially with those nanoparticles *in vitro*. For the formation of protein coronas around 70 nm silica nanoparticles, zebrafish blood plasma was collected separately from female and male adult zebrafish (*Danio rerio*) and the obtained blood plasma was referred to as DrBP-F and DrBP-M, respectively. As previously performed, we firstly characterised each type of nanoparticle-protein complexes as-is and under the exposure condition, and presumed that the main difference between the coronas formed of DrBP-F/M is the biological identity, rather than physicochemical parameters such as colloidal stability. Among the strongly-bound proteins in the corona, female-specific proteins were identified to be vitellogenin, whereas the lack of vitellogenin in the male counterpart seemed to have resulted in preferential enrichment of fetuin. We then exposed primary haematopoietic cells harvested from adult zebrafish to the nanoparticles with a pre-formed corona of DrBP-F or DrBP-M. Interestingly, it was those with a pre-formed corona of DrBP-F that resulted in a higher degree of cellular accumulation *in vitro*, particularly in the myeloid population. What we find striking is that the local build-up of single endogenous proteins at nanoparticles can dictate enhanced immune recognition; in the case of vitellogenin the nanoparticles would otherwise travel along with those proteins bearing the possibility to disturb vitellogenesis. The concept of sex differences in the corona proteome should therefore deserve further attention particularly for oviparous animals.

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Accumulation, speciation and phytotoxicity of pristine and aged silver nanoparticles to wheat

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Silver nanoparticles (Ag-NPs) are used in a wide range of consumer products due to their biocidal action. However, they have been demonstrated to be very easily leached from these products leading to their inadvertent release into the environment. The application of biosolids, in which Ag-NPs leached to sewer systems are retained, as fertilizer in soils, together with the novel use of nanopesticides, makes plants to be significant biological receptors of Ag-NPs. The objective of this work was to compare the induced phytotoxicity as well as Ag distribution and speciation in wheat roots exposed to pristine and aged (sulfidized) Ag-NPs in hydroponics. $AgNO_3$ treatment was used to identify the mechanisms exclusively due to Ag+. By the combination of X-ray Fluorescence (μXRF) and micro X-ray absorption spectroscopy (μXANES), Ag aggregates were identified associated to the cell walls in the epidermis, the cortex and the endodermis of roots exposed to Ag-NP. A process of partial oxidation/dissolution of nanoparticles followed by a progressive chelation to GSH in planta was found in these roots. In Ag_2S-NPs treatment, Ag appeared colocalized with S forming aggregates mainly adhered to the root surface. We found evidence of Ag_2S-NPs reduction to metallic Ag in the root surface. For the plants exposed to $AgNO_3$, Ag was highly accumulated in the root epidermis and also formed spots of several micrometers in the intercellular space of the cortex. Speciation analysis indicated a process of Ag dissolution in the epidermis followed by Ag chelation to thiol and formation of metallic Ag in the root cortex. Phytotoxicity data (thiobarbituric acid production and quantitative real-time polymerase chain reaction) showed that, even if the three Ag treatments induced growth retardation in exposed plants, the toxicity mechanisms that induce this retardation are specific of each Ag source. Toxicity caused by the release of Ag+, represented by the $AgNO_3$ treatment, seemed to be mainly mediated by oxidative stress. Nanoparticles phytotoxicity may be due to both the release of Ag+ and to their high surface reactivity. Overall, the results show the complex interplay between nanoparticulate and ionic form of Ag, and that exposure to Ag nanoparticles induce a complex response possibly due to the multiplicity of Ag species in the plant. Such knowledge on the fate and impact of nanomaterials for plants, is necessary to better evaluate the risk associated to the use of these products.

State of the science on poly- and perfluoroalkyl substances (PFASs) in the environment and humans (II)

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Factors influencing the water-suspended sediment partitioning and sediment levels of legacy and emerging fluoroalkylated substances (PFAS) along the salinity/turbidity gradient of a macrotidal estuary

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Fluoroalkylated surfactants (PFAS) are organofluorine compounds that have been produced and used since the 1950s in numerous applications. Since the global distribution of perfluorooctane sulfonate (PFOS) in wildlife was revealed, PFOS and long-chain perfluoroalkyl carboxylates (PFCAs) have been of particular concern due to their potential for persistence, bioaccumulation, and toxicity, prompting international efforts to phase-out their production or propose more environmentally-sustainable alternatives. Since 2001, the science community has gained a better knowledge as to how long-chain PFASs enter aquatic environments (sources) and partition between water, sediments and biota (fate). However, knowledge gaps still remain to be bridged as regards the environmental behavior of legacy (e.g., PFOS, long-chain PFCAs) and substitution PFAS, especially at the land-sea interface. The question whether PFASs may be scavenged along the salinity/turbidity gradient of a macrotidal estuary is indeed of critical importance to predict their land-sea transport through river discharge into coastal waters. The present survey was conducted in the Gironde (SW France), targeting both legacy and substitution PFASs such as short-chain PFCAs, fluorotelomer sulfonates (FTSAs), and polyfluoroalkyl phosphate diesters (diPAPs). In this estuary, PFASs were ubiquitous albeit at low levels (ΣPFAS < 8.3 ng L^{-1} in the dissolved phase and < 3.3 ng g^{-1} in sediments). In the dissolved phase, ΣPFAS decreased near-linearly with salinity, being PFOS, perfluorohexane sulfonate (PFHxS) and perfluorohexanoate (PFHxA) the dominant congeners. In the maximum turbidity zone (suspended solids up to 2.6 g L^{-1}), the particle-associated fraction was almost consistently > 50 % for long-chain perfluoroalkyl acids (PFAAs), and could be as high as 30–34 % for short-chain PFCAs. Models of K_D partitioning coefficients were derived by integrating both particle-concentration and salting-out effects, and could be used in the future to implement transport models integrating hydrodynamic parameters to estimate PFAS mass budgets at the land-sea interface. Multiple linear regressions taking into account non-detects (data < LOD) provided insights into the relative influence of factors controlling PFAS sediment levels. In that respect, the organic carbon fraction (strongly correlated to sediment grain size) appeared as a more important controlling factor than distance from upstream sources for medium- to long-chain PFAS.

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Perfluoroalkylated Substances in Oceanic Plankton
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Perfluoroalkylated substances (PFAS), including perfluoroalkyl carboxylic acids (PFCAs) and perfluoroalkyl sulfonic acids (PFSAs), are ubiquitous in the environment, reaching the remote oceans, and have been reported to induce harmul effects on terrestrial and aquatic organisms. PFAS bioacumulate and biomagnify in aquatic food webs, and even though there are previous studies on the occurrence of PFCs in biota, few of them report concentrations on the first levels of the aquatic food webs (phytoplankton and zooplankton). The objective of this study was to investigate the global oceanic occurrence of PFCA and PFSA in plankton samples, to elucidate the factors driving their occurrence, and to evaluate their bioaccumulation factors. The analysed seawater and plankton samples were obtained during the Malaspina circumnavigation campaign sampling the Pacific, Indian and Atlantic Oceans, and additionally, water and plankton samples were also obtained in the coastal Southern Ocean at Livingston Island (Southern Shetlands). SPFASs concentrations in ranged from 3 ng g_{dw}^{-1} to 60.9 ng g_{dw}^{-1}, with minimum concentrations in the Pacific and Southern Oceans, and maximum concentrations in the tropical south Atlantic. There was a significant linear correlation between concentrations of SPFAS in plankton and water ($R^2 = 0.45$, p-value< 0.01), which was driven by the two major analytes in this study (PFOS with $R^2 = 0.71$, p-value< 0.01, and PFHxS, $R^2 = 0.63$, p-value< 0.01). Additionally, the isomeric pattern and concentrations of PFOA (n, iso, 5m, 4m, and 3m) and PFOS (n, iso, 5m, 4m, 3m, and 1m) were quantified in plankton samples. PFOA total branched isomers (%br-PFOA) in plankton samples were highest in the Northen hemisphere, while PFOS total blanched isomers (%br-PFOS) followed the oposite pattern. This first report of PFAS concentrations in plankton samples from the global oceans shows that PFASs are ubiquitously found in oceanic plankton, and provides evidence that the concentration of PFSAs is closely coupled between seawater and plankton. In addition, we also provide a first assessment of isomer composition for PFOA and PFOS in plankton in the global oceans. The occurrence and bioaccumulation of PFAS is further discussed in terms of sources, transport and cycling.

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Levels and maternal transfer of PFAS in Arctic hooded seal mother-pup pairs
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Perfluoroalkyl substances (PFASs) are persistent contaminants that are present in the Arctic, and which have the capacity to bioaccumulate and biomagnify in food webs. There is a paucity of studies on the role of milk as a vector for maternal transfer of PFAS, and in particular in marine mammals, which have very lipid rich milk. The aim of the present study was to quantify PFASs in mother-pup pairs of hooded seals (*Cystophora cristata)* and to determine the potential role of milk as a vector in maternal-pup transfer of these compounds in this process. The hooded seal is a high trophic level predator in the arctic marine food web and is therefore susceptible to high exposure of contaminants. This species has a very short and intensive lactation period (3-4 days), enabled by the transfer of extremely lipid rich milk (>60% fat). This makes the hooded seal a good model to study maternal transfer of contaminants. Plasma and milk were collected from 15 mother-pup pairs in the marginal ice zone east of Greenland in 2008, and analysed for PFASs. Perfluorooctanesulfonate (PFOS) was the predominant PFAS in all samples (approximately 40% of ΣPFAS), and the levels were within the range of levels reported in similar studies on pinnipeds. Mean ΣPFAS concentrations were 66 ng/g ww in pup plasma, 36 ng/g ww in maternal plasma and 3.2 ng/g ww in the milk. Higher ΣPFAS levels in maternal plasma compared to the milk supports the concept that binding to plasma proteins limits their incorporation into milk. The individual PFASs differed in their transfer between mothers and pups depending on their carbon chain lengths, with the lowest transfer efficiency for the intermediate chained PFASs and higher transfer of both higher and lower chained carboxylates. The current study was the first to document PFASs in pinniped milk and in hooded seal plasma. We have confirmed maternal transfer of PFASs from hooded seal mothers to pups, and that this transfer likely takes place both via milk and placental transfer; the latter is thought to be the dominant pathway.

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New concern on old substances - endocrine disrupting effects of PFAS
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Concerns for long chain per- and polyfluorinated alkyl substances (PFAS) have been identified due to their persistent, bioaccumulative and toxic properties. Successful regulatory activities are targeting the long chain PFAS due to these properties but also resulted in an increased use of short chain PFAS as “alternatives” by the industry. As a consequence, already today, short chain PFAS are found increasingly in different environmental media. Since they are less bioaccumulative but persistent, they might be spread out in the environment ubiquitously due to their mobility. Therefore, it is of importance to reveal concerns related to their presence in different environmental compartments in order to initiate regulatory measures. Recently, first studies on PFAS endocrine disruptive properties have been published in the scientific literature. Most of the studies using in silico or in vitro test systems. Only limited number of studies has been found in the public scientific literature using in vivo test systems. Hence, most information on endocrine effects could be attributed to the endocrine disrupting mode of action but not to the related adverse effects. While in vitro estrogen receptor binding studies are contradictory for PFAS, in vivo results give the first evidences on estrogen mode of action in fish for some selected perfluoroalkyl carboxylic acids and fluorotelomer alcohols. Several studies indicate interactions of PFAS with the thyroid hormone signaling. Effects assessed by far, are related to the binding of thyroid receptors, thyroid hormone transport proteins and alterations of genes related to thyroid hormone biosynthesis, transport and bioactivation. Unfortunately, aquatic in vivo assays are lacking for the short chain PFAS but the ones available for the long chain members indicating interaction with the thyroid hormone signaling. Only limited number of studies gives first indication on interaction with the glucocorticoid pathway. Although information on endocrine disruption properties of PFAS are mosaic and mostly provided by in vitro studies, the first evidences show that this aspect might require more attention, especially for the short chain PFAS that might be spread out in the environment due to their mobility. Since endocrine disruptors are considered as substances of equivalent concern under REACH, special attention should be given to these properties when assessing short chain PFAS.

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Effects of perfluorooctane sulfonate and alternatives on long-term potentiation in rats in vivo
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With limited but ongoing production and application of perfluorooctane sulfonate (PFOS), the toxicity of its alternatives is lack of evaluation. The present study evaluates the effects of PFOS and its alternatives on long-term potentiation, aiming to provide some evidence for their potential to affect cognitive ability. Targeted alternatives included perfluorohexane sulfonate (PFHxS), perfluorobutane sulfonate (PFBS), and chlorinated polyfluorinated ether sulfonate (F-53B). Different dosages of PFOS and alternative chemicals were given to rats via acute intracerebroventricular injection exposure. The input/output functions (IO), paired-pulse facilitations (PPF), and long-term potentiation (LTP) of the field excitatory postsynaptic potential (fEPSP) ampilitude *in vivo* were recorded. PFOS and alternatives exposure did not significantly affected the normal synaptic transmission, but inhibited LTP in hippocampus CA1 region in a dose-dependent manner. In addition, PFHxS and F-53B exhibit comparable potential to PFOS in disturbing LTP. The results suggested that acute exposure to PFOS and its alternatives resulted in the impairment of the synaptic plasticity by a postsynaptic rather than presynaptic mechanism. Besides, the fEPSP amplitude of baseline was reduced by F-53B but not by other compounds, indicating that F-53B might possess a different action mode. Therefore, further study was conducted to evaluate the effects of the chronic PFOS exposure during developmental period on LTP. The results found that LTP was repressed later in life by early PFOS exposure. Providing some electrophysiological evidence and potential mechanism of the neurotoxic effects induced by PFOS and its alternatives, the present study suggests further evaluation of their safety.

Alternative approaches to animal testing for ecotoxicity assessments

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Comparison of two fish cell lines for the evaluation of model contaminants and PAH complex mixtures toxicity
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Fish are currently used models for the toxicity assessment of chemicals. The REACH Regulation and the European directive on the protection of animals used

for scientific purposes wish the establishment of alternative assays to animal experiments. In this concern, fish cell lines are being developed to provide fast and reliable results on the toxic and ecotoxic properties of chemicals while respecting ethical concerns about animal testing. Rainbow trout liver cell line RTL-W1, which is one of the best characterized cell lines derived from fish for mechanism-specific bioassays and Japanese medaka embryos cell line OLCAB-e3 were used to validate different toxicity assays (MTT, EROD and comet assay) on four model contaminants with different properties: B[a]P, PCB126, Cadmium and Methyl Methanesulfonate. In addition, these cell lines were used to analyze the effects of four polycyclic hydrocarbon (PAH) complex mixtures: *Arabian light* crude oil, refined oil from *Erika*, unleaded petrol and diesel oil. Whatever the bioassays and contaminants tested, dose-dependent effects were observed but with marked differences of sensitivity between cell lines. For the cytotoxicity endpoint embryonic cell line, OLCAB-e3, is more sensitive than trout cell line, RTL-W1 for the majority of compounds. On the contrary, for genotoxicity, the rainbow trout cell line is more sensitive than the medaka one. However, both cell lines give information about toxicity mechanisms of compounds. Our preliminary results confirmed the possibility of using cell lines to assess the toxicity of chemicals, including mixtures, especially in high throughput screening assays. Differences in sensitivity have been found between fish cell lines. It is therefore necessary to carefully select a cell line based on the mode of action and the nature of the studied toxic compound. Same compound will be tested on other alternative to animal experiment: fish embryotoxicity assay. Results will be compared with those obtained on cell lines.

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Fish & Chips: Development of a novel in vitro system of the fish intestine

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Fish are important test organisms in chemical risk assessment. One promising strategy of replacing expensive, time consuming and ethically questionable animal experiments with fish is the use of continuous cell cultures from rainbow trout. Our approach of recreating the intestinal microenvironment of fish *in vitro*, to study physiological responses towards toxicants, is based on the cell line RTgutGC and newly developed microchip technology. *In vivo*, the intestine is lined by a single layer of absorptive epithelial cells, which faces the intestinal lumen on one side and the inner of the body on the other. Herein, the basement membrane represents an ultrathin, highly porous substrate for the epithelial cells. *In vitro*, this delicate membrane is often mimicked with transwell integrated plastic membranes. However, these membranes are relatively thick, show low porosity and visibility of cells using light microscopy is not possible. Using standard microfabrication technology we were able to engineer an ultrathin, highly porous membrane as new cell culture interface. The new membranes are unique in terms of their high porosity and equally distributed nanopores. A single cell seeded on the substrate is in contact with ~3000 pores, while a cell on commercial membranes only faces ~20 pores. A great advantage of ultrathin membranes is their transparency which allows to follow cell growth over time with light microscopy. In addition, confocal microscopy through the membrane results in high quality images. For long term cell culture over 21 days we observed cellular polarization with basolateral actin stress fibers and apical tight junction formation. Non-invasive impedance monitoring over the culture period of 28 days revealed a strong increase in ohmic resistance within the first 24 hours, resulting from cell attachment. Thereafter, resistance increased slowly and steadily due to cell proliferation. Impedance was also used to on-line monitor the response of epithelial cells to toxicants (e.g. sodium dodecyl sulfate). A decrease in ohmic resistance was associated with a decrease in cell viability and disruption of barrier functionality. For ongoing studies we aim to establish a co-culture system of epithelial cells (RTgutGC) and fibroblasts (RTgutF) on chip. Moreover, we will integrate the microchip in a microfluidic bioreactor to allow for realistic, biologically relevant exposure and transport phenomena of chemical pollutants found in the aquatic environment.

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In vitro to in vivo extrapolation of hepatic metabolism in fish: an inter-laboratory comparison of in vitro methods

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Chemical biotransformation represents the largest source of uncertainty in chemical bioaccumulation assessments. Models of chemical bioconcentration in fish may be greatly improved by including biotransformation rates, as measured in vitro. Substrate depletion assays with trout hepatocytes or liver subcellular fractions (S9) have been successfully employed to this end. Regulatory use of these procedures, however, requires that they be evaluated to determine their reproducibility within and among laboratories. Building on previous work, a multi-laboratory ring trial was coordinated by the ILSI Health and Environmental Sciences Institute (HESI). The specific aims of the ring trial were to determine the reliability of these assays within and across laboratories, compare the performance of substrate depletion assays using the two biological systems, and support the development of an OECD test guideline (OECD Project 3.13). Six laboratories (Procter & Gamble, Fraunhofer IME, Dow Chemical, DuPont, KJ Scientific/SC Johnson, Givaudan) conducted substrate depletion assays for 6 test chemicals (pyrene, 4-*n*-nonylphenol, fenthion, cyclohexyl salicylate, deltamethrin, methoxychlor) using both trout liver S9 fraction and trout hepatocytes to determine in vitro intrinsic clearance ($CL_{in\ vitro,\ int}$). For the different test chemicals, the intra-laboratory agreement in $CL_{in\ vitro,\ int}$ averaged 14-22 %CV for the hepatocyte assays and 5-32 %CV for the S9 assays. The inter-laboratory agreement was somewhat higher, ranging from 23- 40 %CV for the hepatocyte assays and 11 -42% for the S9 assays. Extrapolation of the in vitro intrinsic clearance rates for each test system to in vivo intrinsic values resulted in good agreement (within 2x) for all test chemicals except pyrene (~5x difference). Much of the variability in the in vivo clearance predictions was further reduced upon incorporating blood flow limitations and protein binding algorithms into hepatic clearance predictions. As seen in previous studies, inter-laboratory variability was further reduced upon incorporation of in vitro rates into BCF prediction models. The results of this ring trial firmly establish the reliability of these methods and provide strong support for use of this information in modeled BCF assessments for fish.

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Assay development based on the AOP network "thyroperoxidase and deiodinase inhibition leading to impaired swim bladder inflation"

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The vast number of industrial chemicals has generated a strong focus on alternative test development for ecological risk assessment. Therefore, we are developing a non-animal testing strategy for the prediction of chronic aquatic toxicity including *in vitro* tests and *in vivo* ZFET (Zebrafish Embryo Acute Toxicity Test, OECD TG 236) assays. Our assay development process is based on using the adverse outcome pathway (AOP) framework to identify key events (KEs) that could be used to predict chronic toxicity. We developed an AOP network, encompassing thyroperoxidase (TPO) and deiodinase (DIO) activity inhibition, leading to decreased T4 (prohormone) and/or T3 (biologically active hormone) concentrations, impacting swim bladder development and inflation. The latter is considered an ecologically relevant adverse outcome as it affects feeding behaviour and predator avoidance, resulting in lower survival probability. For the assessment of AOP-specific KEs, we optimized *in vitro* assays to characterize the effects of a battery of environmental chemicals with suspected thyroid disrupting activity on TPO/DIO activity. Results were used to predict the impact on swim bladder inflation and validation was accomplished using 120 hours post fertilization (hpf) ZFET and 32 days post fertilization (dpf) FELS (Fish Early-life Stage Toxicity Test, OECD TG 210) experiments. Mercaptobenzothiazole (MBT), a compound identified as a TPO inhibitor, does not directly impair posterior chamber inflation at 120 hpf, while iopanoic acid, a DIO inhibitor, and propylthiouracil, which inhibits TPO and DIO, do. These results affirm our AOP network stating that embryonic TPO activity is not essential for posterior chamber inflation, because of the presence of maternal T4, while DIO activity is needed to activate maternal T4 into T3. However, both enzymes are needed to synthesize and activate THs at later developmental stages, so both TPO and DIO inhibitors were predicted to impair anterior chamber inflation. A FELS MBT exposure indeed resulted in impaired anterior chamber inflation at 21 dpf. Moreover, a relationship between T4 levels and anterior chamber surface further suggests an influence by THs on anterior chamber inflation. *In vitro* assays measuring the TPO/DIO inhibitory potential of compounds were optimized and used to screen a battery of relevant compounds. The results were successfully used to predict the impact on swim bladder inflation, which was validated using ZFET and FELS tests.

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Ecological Threshold for Toxicological Concern (eco-TTC) - Assessing the potential of a new tool for environmental hazard assessment

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The ecological Threshold for Toxicological Concern (eco-TTC) is a new tool under development that summarizes the wealth of ecotoxicological information in the form of Predicted No-Observed Effect Concentrations (PNECs) on diverse chemical substances in the form of statistical (probability) distributions.
Eco-TTCs can be developed that allow prediction of untested chemicals based on structural attributes (categories), mode of action, or functional use. The approach may be useful for assessing chemicals at early tiers of the risk assessment process, provide hazard perspective on chemicals that lack QSARs, can be used to guide product development discussions, and assist read across or category justifications. The proposed eco-TTC is the statistically determined fifth percentile value for the distribution of PNECs for an assessed group. The eco-TTC approach has the potential to reduce the need for animal testing (fish) in many situations. In this talk we summarize the process that has been developed to quantify eco-TTCs and then bring this to life by providing a few examples. Fundamental to the development of eco-TTCs is the formation of a rigorous ecotoxicological database (transparent, high quality studies, traceable to the point of origin). A particularly interesting aspect of this work is segmentation of the data by mode of action and chemical categories. Non-polar and polar narcotic compounds comprise the vast majority of tonnage of chemicals in commerce and provide the first chance to assess the congruence of eco-TTC predictions and patterns with QSARs as well as environmental risk assessments built on empirical data submitted to REACH and other chemical management programs. Approximately 500 chemicals in the eco-TTC database have either a non-polar or polar narcotic mode of action and of these approximately one third have complete acute or chronic data sets (all three taxa). However, the derived PNECs are relatively randomly distributed with respect to application factors applied to the most sensitive taxon when ranked from lowest to largest PNEC. The eco-TTC for non-polar and polar narcotics is explored in depth. Additional category assessments (phenols, esters, reactive compounds, etc.) with sufficient information are under development. Based on the overall data set statements can be made about relative sensitivity of taxa and trophic groups can also be discussed. Eco-TTCs are seen as a promising addition to the toolkit of hazard assessment.

Biomonitoring of contaminants in the marine environment: integration of biological and chemical approaches

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Developing tools for diesel biomonitoring in tropical coastal habitats of Brazil

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Recent offshore petroleum exploration in Brazil has increased the risks of oil spills in tropical habitats. To better protect environmental health, improved oil-spill preparedness and response is needed. In order to identify tools for developing better biomonitoring strategies, we are currently assessing the efficacy of antioxidant biomarkers as post-spill monitoring tools. Our specific goals are 1) to establish a baseline of biomarker values for tropical species 2) to characterize the antioxidant biomarker response in two common species the clam *Anomalocardia flexuosa* and the polychaete *Laeonereis culveri* exposed to sediments spiked with diesel oil under laboratory conditions and 3) to evaluate the biomarker response after chronic exposure to diesel oil *in situ*. Specimens of *A. flexuosa*, the mangrove oyster *Crassostrea rhizophorae*, the snail *Neritina virginea*, the crab *Uca maracoani* and the catfish *Genidens genidens* were sampled in two different seasons, and at locations two locations, one contaminated and another control. Measured markers include CAT, SOD, GPx, GST and LPO. Multivariate analyses were employed to visualize results and a two-way PERMANOVA test was use to test for significant differences in the antioxidant response among species, levels of contamination and seasons. The interaction between season and contamination condition was significant, indicating that biomarker variation responds to complex settings. In the lab experiment, significant differences in antioxidant enzymatic activity depended on the sampling time after the exposure event. Changes in the enzyme activity were fast, with differences occurring between sampling separated only by 24h, proving that the time in which these endpoints are to be measured after a putative oil spill is of major importance. The overall pattern in the enzymatic response was species specific, showing that none of the enzymes measured can be expected to show the same pattern of response (inductive or inhibitory) for both species. A field validation study with chronic diesel oil exposure, to be conducted in early 2015, will include background variability as an informative modulator for oil exposure response at the cellular level. Data provided from this work will contribute significantly to the post-spill monitoring assessment tool-box in tropical waters.

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The marine environment of Kuwait- an integrated monitoring programme to assess the impacts of pollutants

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Threats to the marine environments of Kuwait, and the wider Gulf, are both evident and increasing, and the status of many aspects of the region's unique biodiversity are at record low levels. Like many of the other countries which comprise the Gulf Co-operative Council (GCC), Kuwait has undergone major economic, social and industrial development following the discovery of its oil reserves. This has led to a variety of contaminants being discharged directly to the marine environment, including petroleum hydrocarbons, metals, nutrients, and contaminated brine from desalination plants. Here we report the findings of a comprehensive marine monitoring programme, which along with collecting contemporary data, included an extensive review of a 30 year dataset (water microbial quality, metal pollution and nutrient inputs) to establish the historic baseline of pollutants within Kuwait. A present day assessment of the 'health' of the marine environment, in relation to chemical and biological contaminants, included the analysis of water, sediment and biota for a range of chemical contaminants including polycyclic aromatic hydrocarbons (PAHs), metals, polychlorinated biphenyls (PCBs) and polybrominated diphenylethers (PBDEs). In addition historic inputs of sewage contamination using sedimentary faecal sterol markers were assessed alongside conducting ecotoxicology screening of seawater samples and the assessment of biomarker response and histopathology in key sentinel species. The findings indicate that while Kuwait's marine environment has been subject to a wide array of pollution events, the actual levels of chemical contamination remains relatively low. However, sediment contamination hotspots associated with point sources of industrial contamination were evident at a number of locations around the coast. The assessment of histopathology in fish species indicated that lesions associated with chemical contamination were low when compared with other studies conducted globally. Sewage contamination continues to be a serious issue, with a number of coastal sites heavily contaminated. Linked to this was the observation that seawater samples displayed both acute toxicity and endocrine disrupting potential when screened with bioassay tests. Overall the data allowed 'health assessments' to be conducted at a number of sites and the findings are currently being used by the competent authorities in Kuwait to refine and redesign their national monitoring programme.

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Is alkali labile phosphate (ALP) a suitable biomarker for endocrine disruption in marine mussels?

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Alkali labile phosphate (ALP) is usually measured as a proxy for vitellogenin in fish and molluscs, the ALP method detects inorganic phosphate liberated from phosphorylated proteins like vitellogenin and others. In mussels, it has been measured either in gonad, hemolymph or digestive gland and used in both laboratory and field experiments as a biomarker of endocrine disruption. However, recent studies have shown that vitellogenin is only produced in the female gonad of bivalves and that the phosphorilated proteins measured by ALP in hemolymph correspond to extrapallial protein precursor and not to vitellogenin, that is absent in this fluid (Agnese et al. 2013; Oliveri et al. 2014). This work aims to demonstrate if ALP can still be used as a proxy of vitellogenin in the marine mussel *Mytilus galloprovincialis* when measured in the gonad, the primary site of vitellogenin production and accumulation. For that, several mussels were collected during two seasons representing different maturation stages (early gametogenesis and fully mature), sex and maturation stage were determined by histological analysis and vitellogenin was measured by two techniques: ALP and LC-MS/MS after separation by SDS-PAGE electrophoresis. Protein analysis by LC-MS/MS evidenced the presence of vitellogenin in female, but not in male gonads disregarding their maturation stage. On the contrary, ALP normalized both by protein or by tissue weight, was similar or even higher in males than in females, and did not change with maturation stage. Further protein analyses are currently being done in order to quantify and compare vitellogenin contents in mussel gonad's at different maturation stages and after being exposed to the synthetic estrogen ethinyl estradiol.

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In situ toxicity testing using a nerite gastropod
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Field studies are considered to be more relevant than laboratory tests, as they allow experimentation under natural conditions. Moreover, for some species, endpoints such as reproduction can only be successfully achieved *in situ*. Thus, the aim of this study was to observe the long-term effects of harbor water on a common snail in the Baltic Sea - *Theodoxus fluviatilis*. Harbors (including marinas) are well-known for accumulating pollutants due to poor water exchange. Antifouling paints are one of the main sources of pollution due to their continuous release of biocides into the harbor waters. Thus it is important to assess the sublethal effects of these mixtures of biocides and other contaminants, as they can be an early warning signal for a risk at population level. During two consecutive boating seasons we exposed caged snails in 2 or 3 marinas (2014 and 2015, respectively). The cages were immersed at 1 m depth at all sites and were periodically cleaned to ensure good water exchange. The reproduction of snails was assessed in terms of number of eggs laid in each cage after 2 or 4 weeks. During both years we observed significantly less eggs in all marinas compared to the reference sites (eg between 27- 99 % less eggs). The nutrients in the water were not limiting at any of the sites and therefore we reckon that the reduction in egg-laying in the marinas was due to other factors. Histopathology of the snails is on-going for detecting atrophy of the gonads or any other abnormalities. The marina with the lowest snail reproduction in both years also had the highest mortality, i.e. 6 times higher than at a reference site. The chemical analysis of the water revealed the highest copper and zinc concentrations in the respective marina but further evidence is needed to confirm that these metals are the main cause of the observed toxic effects. Analysis of metallothionein concentration in snails is on-going for measuring a direct biological response to metals such as copper and zinc. This unified investigation of effects at different levels of biological organization will likely help us quantify the contribution of metals vs other organic contaminants to the overall toxicity.

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Biological effects of an offshore oil and gas installation on local fish populations: The Norwegian Water Column Monitoring programme
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The biological effects of an offshore oil platform on local fish populations were assessed as part of the Water Column Monitoring (WCM) programme for 2014. The Njord A platform was chosen as the study location, which was not in operation and had no current discharge of produced water. Demersal fish species were targeted since they were believed to be less likely to migrate away from the platform than pelagic fish. By targeting organisms deeper in the water column and selecting a platform currently not in operation, the impact of drill cuttings and other sediment sources including leakages from well deposits were the main sources of contamination. Wild fish including ling (*Molva molva*), tusk (*Brosme brosme*), redfish (*Sebastes* sp.) and saithe (*Pollachius virens*) were caught with baited rod and line from within the 500 m safety zone of the Njord A platform during the summer of 2014. Reference fish were caught on a separate research cruise by trawling from a region of the Norwegian Sea less impacted by oil and gas activities. Contaminant body burden and a suite of biological effects endpoints were measured in all fish groups and included DNA adducts, DNA strand breaks by comet assay, acetylcholine esterase (AChE) inhibition, ethoxyresorufin 0-deethylase (EROD), vitellogenin (VTG), lysosomal membrane stability (LMS), liver and gill histopathology, PAH metabolites, and PAH body burden. The biomarker data were integrated using the integrated biological response index (IBR/n). Despite low and/or undetected concentrations of PAH and PAH metabolites in fish fillet and bile respectively significant responses in AChE, comet and DNA adducts were found. The biomarker responses indicated exposure to both neurotoxic and genotoxic chemicals in fish inhabiting the lower water column with influence from sediment sources around the Njord A platform. Integration of the biomarker responses (IBR/n) found that all four fish species that were caught from around the platform had markedly higher IBR/n values than their respective reference population. The study shows the advantage of using a suite of biomarkers for assessing the biological effects of low concentrations of complex mixtures with biological effects observed despite low concentrations of PAH measured.

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The Integrated Biomarker Response: a suitable tool to evaluate toxicity of metal-based nanoparticles
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Nanotechnology is a much promising field of science and technology with applications in a wide range of areas such as electronics, biomedical applications, bioremediation, energy, cosmetics, water treatment. Among engineered nanoparticles (ENPs), metal-based ENPs comprise the largest number of ENPs such as silver, gold, copper oxide (CuO), zinc oxide (ZnO), cadmium sulfide (CdS) nanoparticles. The toxicity of metal-based NPs may be either due to their specific physical characteristics as NPs or to the specific toxicity of metals released from NPs under environmental conditions. In this study we evaluated toxicity effects of ENPs (Ag, Au, CuO, CdS, ZnO) and their ionic control part on two endobenthic species: the ragworm *Hediste diversicolor* and the bivalve mollusc *Scrobicularia plana*. The selected exposure concentrations for Cu, Ag, and Cd (10 $\mu g.L^{-1}$) were chosen considering concentrations reported for each metal in highly contaminated marine environments. For Au, because this metal is very inert, the concentration (100 $\mu g.Au\ L^{-1}$) was chosen in order to induce some biological responses. For ZnO NPs, we selected exposure to 3 mg $^{67}ZnO\ kg^{-1}$ sediment since this level is a realistic prediction of the environmental concentration in sediments (Tiede et al., 2009). The implementation of a suite of complementary biomarkers as early warning signals of the presence of contaminants has been successfully applied to provide integrated information on the toxicity of ENPs in aquatic species. A common challenge in multibiomarkers studies is to go beyond an independent interpretation of each one, and to really assess a global response of individuals. The aim of the present study was to test the suitability of the Integrated Biomarker Response (IBR) in both species exposed to the different metal-based ENPs studied or to their soluble metal counterpart to provide efficient and easy tools for environmental managers. We evidence that metal-based NPd lead to an overall difference in biological responses from the control and their soluble counterpart. The IBR could thus be considered as an efficient tool to transfer some research results to stakeholders.

Environmental risk assessment of chemical mixtures: the steps ahead (I)

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Applying mixture toxicity modelling to assess the contribution of detected chemicals to in vitro effects: An example from the Danube River
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Surface water can contain a diverse range of organic micropollutants stemming from a number of sources. Targeted chemical analysis alone is unable to detect all micropollutants present and *in vitro* bioassays can be applied complementary to assess the biological effects of complex environmental mixtures. The aim of this study was to test the hypothesis that the *in vitro* effects in large human-impacted rivers are primarily driven by the mixture effects of many chemicals, rather than being dominated by a particular chemical. In this study a suite of bioassays indicative of activation of the aryl hydrocarbon receptor (AhR), activation of the pregnane X receptor (PXR), activation of the estrogen receptor (ER), adaptive stress responses to oxidative stress (Nrf2), genotoxicity (p53) and inflammation (NF-κB) and fish embryo toxicity were applied to large volume solid phase extracted (LVSPE) samples from the Danube River. This was complemented with targeted chemical analysis of 272 chemicals, with available effect concentrations for the detected chemicals collected from the literature or the US EPA ToxCast database. The contribution of detected chemicals to the *in vitro* effects was assessed using the bioanalytical equivalent concentration (BEQ) concept. Available effect concentrations for between 0 to 13 detected chemicals were found for the different assays. Up to 80% of ER activation was explained by five detected chemicals, with the hormone estrone and the phytoestrogen genistein contributing significantly, while up to 71% of AhR activation could be explained by three chemicals. In contrast, less than 0.2% of the effect was explained by the detected chemicals for PXR activation, oxidative stress response, p53 response and fish embryo toxicity. As a significant fraction of the effect could not be explained by detected chemicals, this study emphasises the importance of applying bioanalytical tools along with chemical analysis for water quality monitoring. The study was limited by the availability of effect concentrations for the detected chemicals and consequently further fingerprinting of water pollutants is recommended.

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Comparison of approaches to assess the risk of micropollutants in Swiss

streams impacted by treated wastewater

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Micropollutants can enter surface waters through various pathways of which wastewater treatment plants (WWTP) and diffuse inputs from agricultural areas play a major role. A multitude of substances are present at concentrations in the ng/l-µg/l range, sometimes close to ecotoxicological relevant values which may induce adverse effects on aquatic organisms. In this study we assess the influence of the coverage of the chemical screening on the predicted risk using different risk assessment approaches and compare it with the observed impact on macroinvertebrates. Grab samples were taken upstream, downstream and at the effluent of 24 different WWTP (12 in 2013 and 12 in 2014). Samples were measured with liquid chromatography high resolution tandem mass spectrometry after offline or online solid phase extraction. Data was analysed for 57 organic micropollutants relevant for Swiss surface waters. For one time point of each campaign an additional comprehensive target screening of over 400 substances was conducted. Risk was assessed with two approaches: i) risk quotient (RQ) approach based on environmental quality standards, and ii) multi-substance potentially affected fraction (msPAF) predicting toxic pressure for aquatic organisms. Comparison with macroinvertebrate data was conducted using the $SPEAR_{pesticide}$ index. The exposure patterns showed expected trends, with higher concentrations downstream than upstream, especially for wastewater borne substances. The chronic RQ of the substance selection was above 1 at more than 15% of the upstream and 95% of the downstream sites. In many cases 4 to 5 single substances explained 95% of the risk; mostly including diclofenac, diuron, and often diazinon. The same substances were in many cases also the drivers of the toxic pressure for the msPAF approach. The comparison between the extended screening data set and the substance selection showed no clear difference using the RQ approach, but increased clearly acute toxic pressure applying the msPAF approach. However, acute toxic pressure remained in the low percentage range (max. 2.3%). The $SPEAR_{pesticide}$ index showed for all sites slightly lower values downstream than upstream and correlated with the fraction of wastewater. The findings indicate an influence of micropollutants discharged from WWTP on aquatic macroinvertebrates. However, the prediction of risk can change substantially depending on the coverage of the chemical data set and the risk approach applied.

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Genotoxic and Ecological risks of river contamination for wild fish populations

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Water pollution by a wide range of substances is considered as one of the main threat to explain fish declines observed worldwide. To reach the good status of European water bodies, the European Water Framework Directive requires the monitoring of the chemical and ecological status of surface water bodies. In this context, biomarkers are considered as complementary to chemical and ecological analysis providing evidences of pollution driven biological defects at the individual scale. In this purpose, the first objective of this workwas to assess a battery of biomarkers on wild sticklebacks (*Gasterosteus aculeatus*). Furthermore, as environmental managers need to know if biomarker responses to environmental contamination are related to ecological risks for fish populations, the second objective aims at studying the relationship between a genotoxicity biomarker response in wild fish germ cells and reproductive impairment. Finally, an integrative approach was investigated gathering three Lines of Evidence (LOE): *i)* chemical analysis, *ii)* biomarker responses as early warning signals of contamination impacting individuals and *iii)* the Fish Based Index as an indicator of fish communities "health status" in French rivers to assess ecological risks for fish populations. Mature male sticklebacks were sampled in sites under contrasted chemical pressures to assess xenobiotic biotransformation, oxidative stress, neurotoxicity and genotoxicity biomarkers. Fish testis were also removed to collect spermatozoa, perform *in vitro* fertilization, assess the level of primary DNA damage in sperm and subsequent progeny survival. Biomarker responses showed correlations between genotoxicity, biotransformation and oxidative stress biomarkers, and discriminate sites under urban pressures. In the contaminated sites, significant lower probabilities of progeny survival were found compared to the reference site as well as a correlation between the level of paternal DNA damage in sperm and progeny survival, thus highlighting potential long-term population defects. Finally, the three LOEs investigated together provide more valuable information to assess ecological risks than using only individual LOE. When chemical and ecological indexes did not draw a clear conclusion, the use of biomarker analyses as the third LOE was relevant to make a diagnostic of the river contamination and of biological impacts.

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Mixture risk assessment for surface waters: Comparative assessment of current approaches for assessing the environmental risk in five Swiss catchments

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On 01.01.2016 a new water protection ordinance enters into force in Switzerland. In addition to introducing environmental quality standards (EQS) for single substances it allows for the consideration of mixture toxicity in water quality assessments as well as the initiation of measures if a surface water body is at risk of being ecotoxicologically impaired by a mixture of substances. Such measures can be taken even if no single substance EQS is exceeded. In the near future a guidance document will be developed that provides simple but sound methods for assessing the environmental risk of contaminant mixtures in surface waters. For this purpose, the current methods for mixture risk assessment were compared using data from a Swiss surface water monitoring study and chronic EQS proposed for Switzerland. The $\mathbf{RQ_{STU}}$ predictions always turn out higher than the simple sum of all individual RQ. Most likely, this is due to the extrapolation of the chronic mixture RQ from acute mixture toxicity predictions by using an AF of 1000. The lowest mixture RQ resulted using a method based on similarity of action ($\mathbf{RQ_{same\_MoA}}$). Since mixture RQ were restricted to substances for which concentration additivity had experimentally been shown, only three mixture RQ groups were assessed: (i) photosystem II inhibitors (triazine and phenylurea herbicides), (ii) inhibitors of very long chain fatty acids (chloroacetanailide herbicides) and (iii) herbicides with auxin action (phenoxy acids). The $\mathbf{RQ_{EQS\_Taxa}}$ method based on identifying the most sensitive trophic level(s) is likely to overestimate the mixture risk if dominant substances have different target taxa within the trophic level. However, the quantitative differences between the methods become marginal, if not all substances present in the sample are analyzed or detected. This was shown by taking passive sampling results for pyrethroids into account, showing that the RQ can be two orders of magnitude higher. All mixture RQ indicate that a risk assessment based on single RQ will understimate the actual risk. However, the current methods for mixture risk predictions still have some shortcomings. For regulatory purposes a combination between the $\mathbf{RQ_{same\_MoA}}$ and the $\mathbf{RQ_{EQS\_Taxa}}$ method seems to be promising. This may define the range of the actual mixture risk. Furthermore, it is important to include highly toxic compounds which are used in the watershed in any risk analysis.

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Using prospective assessment to assess mixtures in municipal wastewater effluents

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Municipal Waste Water treatment plants (WWTP) can be a source of "down the drain" chemicals that are targeted by diverse regulatory agencies concerned with potential environmental effects of these chemicals both independently and as mixtures**.** For the prospective assessment, the chemical mixture is based on domestic activities that are an important source of the chemical mixtures in municipal wastewaters. Starting with activities associated with domestic emissions of chemicals, we identified key products that are used and representative chemicals of those uses. Chemicals were selected based on their reported importance in ecological risk assessments and availability of loading and effects information in Europe. We evaluated a scenario in which a WWTP receives only domestic waste from a fairly small population in order to separate domestic wastewater mixture effects from other influences (e.g., urban stormwater). Chemical loadings to the WWTP were identified based on per capita per day information. Removal rates for each contaminant were based on their physicochemical and biodegradation properties, as well as 3 types of treatment (trickling filter, activated sludge, and advanced oxidation), to determine predicted effluent chemical concentrations (PECs). PECs were compared with predicted no effect concentrations available from the literature. Concentration addition was used to estimate the mixture risk for each scenario. Given activated sludge or better treatment, very few of the representative chemicals individually presented a risk to aquatic life even when PECs were increased over 10-fold. Given the chemicals used in this analysis and a concentration addition approach, mixture risk for the activated sludge treatment with or without the addition of advanced oxidation was driven by very few chemicals: galaxolide, EE2, sulfomethoxazole, and carbamezapin. These chemicals have different modes of action and when evaluated using response addition indicated little or no mixture risk given this analysis. Trickling filter treatment resulted in much greater potential for mixture risk in our analyses. Scenarios such as those used here can provide testable hypotheses for studies that include the use of biomonitoring or retrospective-based mixture assessments.

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Predictability of the Aquatic Toxicity of Realistic Mixtures in Wastewater

Effluents and Their Risk Assessment
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The concept of Concentration Addition (CA) is widely accepted as a default approach for predicting combined toxicity of mixtures of chemicals. However, it can only be applied to mixtures of clearly defined components for their clearly defined mass proportions in the mixture, which limits its applicability to a routine risk assessment of highly complex and variable wastewater effluents. In order to explore the relevance of a mixture assessment compared to a single-substance assessment of residues in wastewater effluents, mixtures of up to ten substances were assessed at two different concentration ratios, including the experimental verification of their predicted toxicity in the absence and presence of wastewater background. The mixtures were tested at an effect-based ratio and at an exposure-based ratio that resembled a realistic mixture scenario. The toxicity of the mixtures could be reasonably well predicted for chronic endpoints both in green algae and in *Daphnia magna*. There was rather a slight tendency to overestimate mixture toxicity by the CA concept than to underestimate it, which demonstrates that the CA approach would be predictive for a mixture risk assessment without being over-protective. The predictability of the mixture toxicity was not influenced by wastewater effluents, i.e. by the presence of other substances at very low concentrations. The presentation will also compare preliminary risk assessments based on measured environmental concentrations (MEC) and predicted environmental concentrations (PEC) and on chronic endpoints of single substances as well as mixtures together with assessment factors for each test organism. In addition, preliminary predicted no effect concentrations (PNEC) will be derived for single substances and the mixtures, and they will be compared to summed MEC and PEC, which will demonstrate the difference for such mixture assessments conducted seperately for each trophic level or across trophic levels.

Fate and Effects of Metals: Regulatory and Risk Assessment Perspective

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Retrospective monitoring of butyltin in fish and mussels from German marine and freshwaters
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Tributyltin compounds (TBT) and their degradation products dibutyltin (DBT) and monobutyltin (MBT) have been proven to have strong ecotoxicologically effects on aquatic organisms (e.g. imposex of neogastropods, shell malformations of mussels). Therefore, a ban of TBT-containing antifouling coatings for small vessels under 25 m was imposed by the European Commission in 1989 (89/677/EWG) as a first measure. Since 1st January 2003, the usage and distribution of antifouling preparations containing organotin compounds is prohibited by the Commission Directive 2002/62/EG. Furthermore, in 2008 this ban was extended to EU-foreign flagged vessels. A retrospective monitoring of organotin compounds (including TBT, DBT and MBT) in archived freshwater and marine biota samples from the German environmental specimen bank (ESB) has been carried out earlier. The time series for butyltin concentrations in muscle tissue of freshwater fish (bream, *Abramis brama*) and marine fish (eelpout, *Zoarces viviparus*) as well as in marine mussels (*Mytilus edulis)* tissue were extended from 2003 to 2011 and samples from 2012/2013 are under investigation. The marine samples were collected from the North and the Baltic Seas, and the freshwater samples from the German rivers Elbe, Danube, Rhine, Mulde, Saale, Saar as well as from Lake Belau. After digestion, derivatization and extraction, trace levels of organotin compounds in the pooled and homogenized biota samples were analyzed by a validated sensitive SID-GC/ICP-MS method (species specific isotope dilution - gas chromatography - inductively coupled plasma-mass spectrometry). The results of this project allow answering the following questions: 1) Does the ban of organotin compounds as antifouling agent show positive effects on the butyltin concentration in aquatic biota? 2) Are there any differences in concentrations between the various sample sites and the various biota samples? 3) Are there any temporal trends in the concentrations of TBT, DBT and MBT?

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Determination of water quality criteria for uranium that account for bioavailability in freshwaters
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Uranium (U) is a chemo- and radio- toxic substance of concern for the aquatic environment, with potential releases to freshwaters due to e.g., mining, processing and waste disposal. In France, a 'generic' Predicted No Effect Concentration (PNEC) for freshwater ecosystems has been set to 0.3 μg/L of dissolved U in the water column in addition to the background, based on an exhaustive review of available ecotoxicity data and the use of assessment factors. However, as many other metals, it is well known that U toxicity depends on its bioavailability which is in turn mainly driven by its speciation. For such elements, the last European guidance for deriving Environmental Quality Standards recommends now explicitly to take into account their bioavailability. Therefore, we developed a method based on theoretical simulation of U geochemical speciation in freshwaters (VMinteq v3.0, considering U complexation with dissolved organic matter and with alkaline earth and carbonate ions). Based on the assumption that the bioavailable species of U were UO_2^{2+}, $UO_2(OH)^+$, $UO_2(OH)_2$ and UO_2CO_3, a statistical regression tree analysis was performedto identify physico-chemical domains representative of French freshwaters where different PNEC might apply. Combining these first results to the available chronic U ecotoxicity data for each physico-chemical domain identified allowed us to derive four PNEC values (ranging from 0.3 to 30 μg/L) conditional to four physico-chemical domains that differ by their alkalinity (cut-off at 75 mg HCO_3^-/L), pH (cut-off at 7.5) and DOC content (cut-off at 6 mg/L). These findings were formalised in a determination tree. Further analyses were performed to evaluate the robustness of this tree and its domain of applicability. These conditional PNEC values for freshwaters could be used as a refined step in any ecological risk assessment for uranium. However, their applicability in any monitoring programme would need some adaptation in the monitoring practices: not only the dissolved fraction of uranium, but also the influent physico-chemical parameters (pH, alkalinity, DOC) should be measured together, with a sufficient accuracy and sensitivity.

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A new approach to diagnosing the ecological effects of metal-contaminated sediment in natural systems
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Pollution from abandoned metal mines is a serious impediment to rivers meeting the water quality targets set out by the EU Water Framework Directive. Recently, there has been recognition that managing these risks requires approaches to setting environmental guidelines that are based on a consistent definition of exposure in systems where bioavailability may vary, thus allowing more robust and useful ecological effect thresholds to be identified. We have used a new approach to relate the levels of toxic metals in sediments affected by mining to ecological damage based on bioavailability. The concept is to use widespread biomonitor species as chemical probes for bioavailable metal, and to relate this measure of metal stress to variation in invertebrate community composition (a WFD-compliant measure of ecological condition). This approach bridges two established methodologies that are traditionally used independently (community-level field observations and laboratory toxicology). We selected 99 river sites in England and Wales, across 20 spatially-independent, replicate river catchments impacted by metal mining. Within each catchment, sites were located upstream and downstream of abandoned mine facilities. At each site we sampled the macroinvertebrate community, collected biomonitor specimens for metal bioaccumulation analysis, and collected a fine sediment sample for metals content, organic carbon and particle size analysis. Using multivariate ordination techniques we then quantified the response of the macroinvertebrate community to variation in metal bioavailability, having first taken into account the confounding effects of other environmental factors. We identified the position of taxa across the gradient metal bioavailability, providing the basis for a new diagnostic metric to metal stress in rivers. The new metric was tested on an independent dataset drawn from existing data sources. Our aim is to provide a WFD-compliant method for detecting where contaminated sediments are causing ecological impacts.

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Ten years on: Soil screening values for ecological risk assessment in the UK
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A presentation on the "Use of soil screening values for metals for ecological risk assessment in England and Wales" was made at SETAC Europe in 2006. Since then, both scientific knowledge and regulatory priorities have changed in the UK in regards to soils and ecological protection. This presentation, 10 years on, provides an outline of those key changes and demonstrates how the continued practical and pragmatic application of complex scientific concepts can deliver solutions to regulatory challenges that face many in Europe and across the globe. The previous presentation focussed upon trace elements and in particular the implementation of a bioavailability framework to derive soil screening values (SSVs), to provide a generic screening tool to 'rule out' the further assessment of potentially contaminated sites. Just 10 soil screening values (SSVs) for prioritised chemicals were derived at the time. Regulatory priorities have now changed from contaminated land, to assessment wastes destined for recovery to land. SSVs are needed for many more and different chemicals that 10 years ago. However,

through the use of new guidance, the ECHA dissemination portal and industry collaborations SSVs have been devloped that utilise new data and research findings. This presentation will show that the lag between scientific developments and regulatory implmentation can be relatively short. Changing regulatory needs can keep pace with scientific developments. \n

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Assessing the integrated terrestrial ecotoxicity of Cu and Ni species for life cycle assessment (LCA)

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Life cycle assessment (LCA) validity of metals toxicity can be significantly affected by bioavailable metal fraction. The bioavailable fraction of Copper (Cu) and Nickel (Ni) for world soil types are calculated using WHAM 7 geochemical speciation model. Applicability of WHAM model for soils using their globally available properties (texture, pH, cation exchange capacity, and organic matter content) is examined and verified with soil field data and compared with an empirical regression method. The results obtained from WHAM 7 show that the model is able to calculate bioavailable fraction of Cu and Ni with uncertainties of less than two and three orders of magnitude, respectively, for a wide range of soils distributed globally. It is in better match with the experimental data in term of absolute value than the empirical regression results. To address this variability, 518 archetypes and 13 groups of archetypes are created to show the global distribution of obtained BF values. Moreover, BFs ranking was compared and the soluble Cu and Ni Spearman rank correlation coefficients showed a significant correlation between BFs obtained by WHAM and BFs calculated by the regression method. The BFs included in the calculation of CFs for Cu and Ni have been applied in terrestrial ecotoxicity and the level of regionalization required to adequately represent the spatial variability of CF has been defined. The obtained results from two different versions of the model (WHAM 6.0 and 7) were also compared for Zinc (Zn) to see whether the updated database of the model may change the results. This study validates using WHAM as a tool to study Cu and Ni bioavailable factor in global soils since the results cover a considerable range of world soils. This research will not only obtain regionalized CFs including Cu and Ni speciation in terrestrial ecotoxicity directly applicable in the current LCA methods, but also will show that it is possible to use geochemical models for large scale speciation in soil. We applied WHAM 7 for Cu and Ni to determine the BFs in soils to validate this approach with measured speciation data in term of absolute value and ranking and to be sure our big effort of modelling with warm is worth it. We showed it brings more appropriate answer than the regression method.

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Quantitative risk assessment of mercury contamination to ecosystem in a mercury mining area

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Wanshan Mercury Mining Area (WMMA) which is located in Southwest China has a long history of Hg exploration and smelting. Hg contamination has been a serious local problem for both human and environment for decades. The risks of Hg for human health have been reported in WMMA by several studies, and dietary intake rather than inhalation has been identified as the main exposure pathway for the local residents. However there has been less focus on the risk of Hg contamination on the ecosystem. The scope of this study is therefore aimed at quantifying the risk related to Hg pollution from an ecosystem perspective. The endpoints of the assessment will be plants and biota rather human. This study aims at developing a site specific index system to identify the risks of Hg contamination on major biota species (rice, invertebrates and birds). The exposure level in this study is determined by direct sampling and measurement of Hg concentrations in different references species at WMMA (soil, rice, earthworm, grasshopper, spider and bird samples were collected). Total Hg concentration is selected as proxy for comparing the concentration levels. No adverse effect concentration (NOAEL) is used as the risk assessment benchmark for the different species. Risk quotients (RQ) are then derived based on comparing the Hg concentrations in each species with benchmark values. Four levels of risk were proposed based on RQ values: Level 1: Background. If all RQ values are smaller than 1, and RQ are smaller than reference site, the species is then considered to be living under a similar condition as in the background site. Level 2: Minimal risk. If all the RQ values are smaller than 1, the species is then considered having minimal risk. Level 3: Moderate risk. It is possible that RQ of tissue concentration is smaller than 1, but RQ of diet is bigger than 1. This indicates that the species is having a risky diet, while tissue concentration has not reached risky level. Level 4: High risk. If RQ based on tissue concentration is greater than 1, it can be confirmed that the species is at risk. The plan for remediation at WMMA is under development with local authority, the outcome from the risk assessment will then supply important basis for the remediation method development, and possible measures to reduce the risk will be then proposed based on the results.

Higher tier tests in the risk assessment of plant protection products (II)

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An SSD-based investigation of the relative sensitivity of Daphnia magna - Implications for risk assessment

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The species sensitivity distribution can be used to quantify the spread in sensitivity between different organisms. A large width of a distribution implies that there is a large difference between the most and the least sensitive species tested. Currently approximately 30% of the PNEC's from the single substances registered under REACH can be traced back to *Daphnia magna*. In order to determine whether the current risk assessment practice is protective, we compared the sensitivity of *Daphnia magna* to the sensitivity of other aquatic invertebrates. Ecotoxicity data were initially collected from the US EPA database ECOTOX. The data was thereafter filtered and only those compounds that contained acute toxicity data (1-7 days) from laboratory studies performed on aquatic invertebrates were kept. Finally the species sensitivity distributions were fitted using the R package "drc" for all compounds where 8 or more species had been tested. In total 157 species sensitivity distributions could be generated. The results show that the sensitivity of *Daphnia magna*, in relation to other tested aquatic invertebrates, varied randomly. The median factor between the most and the least sensitive aquatic invertebrate for each individual compound is estimated to be around 1300. The species sensitivity distribution for three specific compounds show that 2.3% (Temephos), 46.4% (Toluene) and 93.75% (Endosulfan) of all aquatic invertebrates are assumed to be more sensitive than *Daphnia magna*. The difference between the relative sensitivity of *Daphnia magna* for these three compounds demonstrates the necessity in comparing the available species sensitivity distributions to the currently used assessment factors within chemical risk assessment.The presentation will provide further details on the spread of the species sensitivity distributions and the distribution of sensitivity steepnesses over all the tested chemicals will be analysed.

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EFSA Scientific Opinion on the effect assessment for pesticides on sediment organisms in edge-of-field surface waters

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Some aspects, proposals and/or recommendations described in the EFSA scientific opinion on the effect assessment for pesticides on sediment organisms in edge-of field surface water are presented here. They include elements of exposure as well as effect assessments since the ERA is a combination of both. The issues highlighted here are the following: Triggers for sediment ecotoxicity testing and a decision scheme based on a tiered effect assessment approach for different types of organisms; How to link regulatory acceptable concentrations (RACs) to predicted environmental concentration (PECs), using the ecotoxicologically relevant concentrations (ERCs) concept that is influenced by the choice of sediment layer depth, exposure metric and test duration; Methodology for introducing an accumulation factor to account in a conservative way for effects of multi-year applications; Assessment of impacts of bioaccumulation, biomagnification and secondary poisoning; Issues related to uncertainties of the current and/or proposed ERA approaches. The specific protection goals (SPGs) for sediment-inhabiting organisms are based, as for the aquatic organisms in edge of field waters, on two options, i.e. (1) the ecological threshold option (ETO), accepting negligible population effects only, and (2) the ecological recovery option (ERO), accepting some population-level effects if ecological recovery takes place within an acceptable time period. For several reasons, for the time being it is suggested that the ETO is the best option to provide adequate protection of benthic organisms.

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Effects of sediment-spiked lufenuron on benthic macroinvertebrates in single-species tests and outdoor microcosms

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Sediment ecotoxicity studies were conducted with lufenuron, a hydrophobic benzoyl urea insecticide. The studies aimed to compare the concentration-response relationships for macroinvertebrates observed in an outdoor sediment-spiked microcosm experiment and those of 28-d sediment-spiked single-species toxicity tests with *Chironomus riparius*, *Hyalella azteca* and *Lumbriculus variegatus*. Twelve weeks after the construction of the microcosms mean measured exposure concentrations in the sediment were on average 87.7% measured initially. Treatment-related effects of sediment-spiked lufenuron in the microcosm experiment were observed for populations of insects and macrocrustaceans (lowest NOEC for benthic insects 0.8 µg a.s./g OC and for benthic crustaceans 8.5 µg a.s./g OC). Treatment-related increases in abundance (indirect effects) were observed for benthic oligochaete worms (lowest NOEC of 0.8 µg a.s./g OC). The 28-d sediment-spiked laboratory toxicity tests resulted in EC_{10} values of 0.49 µg a.s./g OC for *C. riparius*, 1.20 µg a.s./g OC for *H. azteca* and 211 µg a.s./g OC for *L. variegatus*. These data also explain why oligochaete worms did not suffer pronounced toxic effects in the microcosm experiment, but could increase in abundance due to the decline of sensitive benthic arthropod populations (release of competition). The chronic laboratory toxicity data mentioned above illustrate that the tier-1 effect assessment approach proposed by EFSA for benthic organisms and sediment-bound insecticides, using the lowest chronic EC_{10} value for the combination *C. riparius* and *H. azteca* and an assessment factor of 10, is protective for the insecticide lufenuron and the responses observed in the sediment-spiked microcosm test.

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Applying the MDD concept to terrestrial NTA studies

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Natural terrestrial ecosystems such as meadows adjacent to cropped fields may be exposed to spray drift. The arthropod fauna of such ecosystems is generally abundant and species rich and comprises a broad array of eco(toxico)logical profiles. There are no models or model systems sufficiently fit to adequately predict the potential effect of exposure on the functioning of these ecosystems. For this reason experimental tests are performed, generally in natural ecosystems without prior manipulation. Because the arthropod fauna in such systems varies between locations and years there is a need to evaluate whether a selected site is appropriate for the purpose of the experiment, i.e. to derive regulatory acceptable exposure levels. This can only be done a posteriori. We apply the MDD concept derived by Brock et al. (2015)[1] for aquatic micro-/mesocosm studies to natural (i.e. unbounded) terrestrial off field systems as described in DeJong et al.[2]. Typically these experimental systems follow a replicated (n=4) plot design, with 30x30 m plots and multiple sampling methods over a period of 8 weeks following a single application event. A large number of arthropod taxa (800-1000) is assessed and typically at least 80-100 are sufficiently abundant for statistical hypothesis testing. As a consequence of inherent parametric constraints we use non-parametric tests for these analyses. Our primary objective is to test whether the criteria in the aquatic scheme can be applied to derive regulatory acceptable exposure levels for terrestrial systems as well. We use the decision scheme in Brock et al. (2015) to data obtained in a large number of off-field studies. MDD's were calculated using the technique provided by Van der Hoeven (2008)[3]. Potentially sensitive taxa were defined using empirical criteria, in particular whether a specific or related taxon was consistently affected by the reference treatment in different studies. Ecologically vulnerable taxa were defined similarly as taxa consistently affected by the reference item for four or more weeks in different studies. [1] T.C.M. Brock; M. Hammers-Wirtz; U. Hommen;T. G. Preuss; H-T. Ratte; I. Roessink; T. Strauss; P.J. Van den Brink. Environ Sci Pollut Res (2015) 22:1160–1174. [2] F.M.W. de Jong; F.M. Bakker; K.Brown; C.J.T.J. Jilesen; C.J.A.M. Posthuma-Doodeman; C.E. Smit; J.J.M. van der Steen; G.M.A. van Eekelen. 2010 ISBN/EAN: 978-90-6960-245-5 [3] Van der Hoeven, N. 2008. Ecotoxicol.Environ.Saf. 70:61-66

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Development of suitable experimental designs for semi-field trials with solitary bees

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The publication of the proposed EFSA risk assessment of plant protection products for pollinators led to an increasing demand for experiments with non-*Apis* pollinators. However, no official guideline for the standardized semi-field trials exists so far. To overcome this lack of guidance, a semi field study was performed to continue our research and increase the knowledge about suitable test designs and the handling of the test organisms. The aim of this study was to implement a test system for trials under semi-field conditions with solitary wild bees. In the study the potential effects of exposure of bees and their brood to test item treated and untreated plants and the statistical evidence of validity were the focal points. During the exposure and after end of the exposure all relevant parameters were recorded. The test design is in accordance with a proposal from the ICPPR non- *Apis* working group. The semi-field study with the red mason bee *Osmia bicornis* (Hymenoptera, Megachilidae) was conducted in winter oilseed rape with 6 replicate tunnels per treatment group and contained a water treated control as well as two reference treatments applied with 100 g a.i./ha and 350 g a.i./ha dimethoate. The following end points were observed in the study: nest occupation by female individuals, flight activity, reproduction capacity by means of produced cells and cocoons and brood termination rate. Hatching success was also recorded to assess the viability of the used test specimens. The first results show clearly the possibility to perform semi-field studies with the red mason bee *Osmia bicornis* in winter oilseed rape. Dimethoate can be used as a toxic reference to show acute effects on adult wild bees. The endpoints chosen were useful for a study design and the variability was low with regard to the observed effects. The two rates of dimethoate tested showed already the maximum effect so that dimethoate can be used as a toxic reference in semi-field studies at the lower rate. Furthermore the statistical analysis showed that the test design is valid and repeatable.

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Experimental design for semi-field trials to test brood affecting plant protection products with solitary bees

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The newly proposed EFSA risk assessment of plant protection products for pollinators includes for the first time not only honey bees as test organisms but also non-Apis pollinators. However, there is no official guideline for standardized semi-field trials. To support a tiered risk assessment a semi-field study design was developed and performed in 2015 based on available publications and advices from an ICPPR workshop. The results of these studies make it possible to improve future designs and recommendations for the handling of the test organism can be given. The objective of this study was to develop a semi-field test design for plant protection products affecting brood of solitary wild bees. In the study the potential effects of exposure of adult bees and their brood to an insect growth regulator were examined. After the end of exposure the development of the progeny was followed until the following spring and the reproduction success was evaluated as an endpoint. The semi-field brood trial with the red mason bee *Osmia bicornis* (Hymenoptera, Megachilidae) was conducted in a *Phacelia* crop. The test design included a water treated control and two treatment groups. Each treatment group was replicated with 4 tunnels. The exposure period started at the beginning of July 2015. The treatment applied was fenoxycarb, an insect growth regulator also used as a reference substance in honey bee brood studies. Two rates were tested with150 g a.i./ha (T1) and 350 g a.i./ha (T2). The following endpoints were observed in the study: to evaluate sub-lethal effects on adult bees, the nest occupation of females and the flight activity was documented. I order to evaluate brood effects, the cell production, the cocoon production and the brood termination rate were assessed. To ensure equal starting conditions in all treatment groups the hatching success was recorded. The first results show, that it is possible to perform a semi-field brood study with the red mason bee *Osmia bicornis* in *Phacelia*. The bees can be stored until June and hatched specimens are still viable and fertile. Fenoxycarb can be used to introduce brood termination in eggs and larvae of red mason bees. The endpoints chosen are useful to evaluate effects on reproductive success and the variability between replicates was low. The lower rate of fenoxycarb showed already the maximum effect so that fenoxycarb can be used as a toxic reference in semi-field studies.

Methodological challenges for LCA of agricultural supply chains producing food, fibre and bioenergy

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Comprehensive assessment of fruits and vegetables human health effects in a LCA context

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Purpose: Nutritional effects from the 'use stage' of food items life cycle can have a substantial effect on human health; yet, they are often not considered in life cycle assessment (LCA). In our study we explore the case of increased fruit and vegetable consumption, a healthy dietary option – that could result in higher exposures to a wide variety of pesticides – and investigate the trade-offs between associated environmental and nutritional health effects. *Methods:* We employ the Combined Nutritional and Environmental Life Cycle Assessment (CONE-LCA) framework that evaluates and compares in parallel the environmental and nutritional effects of foods expressed in Disability Adjusted Life Years (DALYs). For the environmental health assessment we consider impact categories such as global warming and particulate matter (PM) as well as chemical exposure due to pesticide residues. Global warming and PM are assessed following a traditional LCA approach. For the pesticide residue exposure, we use publically available health impact scores derived from toxicological studies of numerous pesticide active ingredients. For the nutritional assessment we focus on the various health outcomes considered in the global burden of disease that are based on epidemiological studies. *Results and discussion:* Adding one serving of fruits or vegetables to the current average diet in Europe may lead to substantial nutritional health benefits. These nutritional benefits are slightly increased when we consider substitution scenarios in which the substituted food items are associated with negative health effects, such as red meat and trans-fat. Overall environmental health impacts associated with this addition are substantially smaller compared to nutritional benefits in each scenario, even when considering an uncertainty factor of 400 for the impacts of pesticide residues. *Conclusion:* The present study illustrates the importance of considering nutritional effects of food items in LCA. Our preliminary results suggest that nutritional health effects of food items can be substantial and comparable to environmental impacts, especially for nutritional foods such as fruits and vegetables. This approach could be used for making recommendations about sustainable diets and food choices.

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Pesticides' impacts of bananas from different regions

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Most bananas sold in Europe nowadays have some sort of sustainability label. Consumers and supermarkets therefore may have the idea that the labelled bananas are produced in a sustainable way. Sustainability covers many social and environmental themes and several of these are addressed by the labels only to a certain extent. However, large amounts of pesticides are still used in conventional banana production, resulting in potential human health and ecosystem impacts. Using no xenobiotic pesticides at all as is the case for organic bananas is not an option for the large scale, because it needs very specific climatic and logistic conditions. Different climatic, soil conditions and production practices between farms result in large differences in the pesticide impact of bananas. In this study, the toxicity-related impacts of pesticides applied in banana production were calculated with the consensus model USEtox version 2.0 for three case studies: an organic farm in Peru, smallholder farms in Ecuador, and a conventional plantation in Panama. The resulting toxicity-related pesticide footprints show that the use of large quantities of myclobutanyl, chlorpyrifos and mancozeb in Panama case have the highest contribution to the human health impact profile. The use of azoxystrobin and mancozeb in this case shows the largest share on the impact profile for organisms in freshwater ecosystems. The footprint of the pesticides used in banana production from the Panama case is about 20 to 30 times larger than in the Ecuadorian case. In a sensitivity analysis, in each case, regionally specific landscape parameters were used to calculate specific factors for each of the pesticides used. This increased the score of the Panama case by about 300%, while the score of the Ecuador case did not change significantly. Default values for these parameters are provided by USEtox for several regions, among which for a region covering Central America and one covering Peru and southern Ecuador. Calculations with an alternative impact assessment model (USES-LCA) generally confirms the results with USEtox, but shows that the impact on terrestrial and marine ecosystems, which are not included in USEtox, can be significant.

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Towards a consensual method to assess climate change impacts from bio-based systems

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This study focused on climate change impact assessments of systems involving compartments of the biogenic carbon cycle, addressing the issue of assessing the actual impacts of biogenic carbon emissions and of variations in carbon stocks. A critical review was carried out on seven different characterisation models dealing with these environmental mechanisms, including five methods dealing with GreenHouse Gas (GHG) emissions - conventional Global Warming Potentials (GWP) from IPCC according to a carbon neutrality approach or to a full accounting approach, time-adjusted GWP, biogenic GWP and biogenic accounting factors - and two methods dealing with land occupation and/or transformation - ILCD / IPCC recommendation to account for carbon stock changes from land transformation and Müller-Wenk proposal adopted in the land use framework. These models were rated over eight criteria divided into four categories: completeness in terms of environmental mechanisms covered, scientific soundness, genericity, and easiness of use. Results showed that the currently recommended methods are conventional GWP according to a full accounting approach for the assessment of greenhouse gas emissions, and the ILCD / IPCC recommendation to account for carbon stock changes from land transformation. However, despite their good international acceptance, these methods fail to take into account the dynamic nature of the biogenic carbon cycle and new methods have then been developed for this purpose. These methods present many gaps but two were identified as promising characterisation models: time-adjusted GWP and Müller-Wenk proposal. Finally, a new method that benefits from the advantages of these two last methods is proposed. This method relies on the land use framework related to carbon sequestration potential with the time-adjusted GWP embedded to better reflect the dynamics and reversibility of the biogenic carbon cycle. It is compatible with full accounting approach for GHG emissions and temporary carbon storage valuation. It also remains sufficiently practicable.

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Which functional unit to assess environmental impacts of dairy system intensification?

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Intensification of milk production, defined as increased production per hectare (ha) of land, invariably leads to increased impacts per ha, but its impacts per kg of milk are less clear. The aim of this study was to assess a range of environmental impacts of contrasting dairy systems that represent a wide diversity of management practices and intensification levels. We used the concept of the Technological Management Route (TMR), i.e. a logical set of technical options designed by farmers, to compare seven systems representing the diversity of milk production systems in France and a range of intensification levels. Life Cycle Assessment was used to estimate impacts of these systems using two functional units (FU): t of milk and ha of total (on- and off-farm) land occupied. With the area-based FU, we are looking for low-impact land-use systems. From this perspective, the organic and highland systems were most promising. With the mass-based FU, we consider productivity and impacts. From this perspective, a maize-silage based system seemed most promising, as it ranked lowest or second-lowest for six out of seven impacts. Dairy system intensification had three effects: i) all impacts increased per ha of land occupied, ii) eutrophication and land competition decreased per t of milk produced, and iii) other impacts changed little per t of milk produced. In other words, depending on the FU, the perceived environmental impacts of dairy system intensification differed radically. A mass-based FU is by far the dominant FU, and, in studies that go beyond the farm gate, the only FU used. Thus, current LCA practice is largely blind to environmental impacts of dairy system intensification, as it tends to ignore the environmental impacts of intensification revealed by the area-based FU. This is a sobering observation with paradoxical consequences, as this “blind spot” of current LCA practice may well tend to bias decision making in favour of intensive systems, which have high impacts per ha of land occupied. LCA-based decision making might thus increase the prevalence of intensive systems, which could increase overall impacts of the agricultural sector. Reconciling environmental impacts and productivity is difficult. Using only a mass-based FU, does not provide a balanced view of the impacts of intensification and could mislead decision makers in identifying promising dairy systems. We recommend the use of both mass-based and area-based FUs in LCAs of agricultural goods.

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Environmental Impact of food consumption in EU

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In order to comprehensively assess the impact of food consumption at EU level, a detailed product based LCA from ‘cradle to grave’, has been conducted aiming at: i) identifying the most representative food and beverage products consumed in the EU-27 via a statistical analysis of food consumption, selecting 2010 as reference year, ii) evaluating, via an LCA, the life cycle environmental impact of the average food consumption of an EU-27 citizen in one year following the ILCD recommendations for impact assessment, iii) developing a strategy for using the BoP food as baseline scenario for testing ecoinnovation options for impact reduction. The methodology developed for assessing the impacts of food consumption in EU, based on a basket of food products, includes the following steps: 1) Quantitative and qualitative analysis of the structure of the EU consumption category of nutrition – during the years 2000-2010 – including international trade and selection of a basket of representative products for the consumption category of nutrition for the year 2010. 2) Collection and development of process-based LCIs for the selected representative products. 3) Calculation of the environmental impact results, based on the results of the previous steps. 4) Quantitative and qualitative analysis of the environmental impacts of the selected nutrition basket, with conclusions and recommendations for the future. The overall results indicate that in the majority of the impact categories the most burdening consumed foods are meat products and dairy products. The agricultural phase is the most impacting lifecycle stage of the basket food, due to the contribution of agronomic and zoo-technical activities. Food processing and logistics follow in importance, due to their energy intensity and the related emissions to atmosphere, occurring during the production of heat, steam and electricity and during transport. Regarding the end of life, human excretion and wastewater treatments are posing burdens related to euthrophying substances. The impact assessment results of this study could be used as a means to provide an index for monitoring and analysis, in order to evaluate the effect of possible improvements within the life cycle stages and the different supply chains. A further step of the analysis should be to develop scenarios of eco-innovation and behavioural changes to test their effect at the EU scale and to prioritize their implementation.

Persistent and mobile contaminants in the aquatic environment: how to identify, analyse and regulate a

potential threat for drinking water resources (I)

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Identification, analysis, removal and regulation of persistent and mobile organic chemicals in the drinking water cycle - The approach of the EU project PROMOTE
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Surface water and groundwater are the two major sources for drinking water in Europe. Their chemical quality may be affected by human activities, among them the release of chemicals that we are producing and using. If these chemicals are poorly degradable (persistent) and polar (i.e., mobile in the aquatic environment), or if they are transformed into poorly degradable and polar compounds, then these chemicals may specifically be of concern with respect to contamination of water used for drinking water production and, eventually, for the quality of drinking water itself. We denote such compounds persistent mobile organic chemicals (PMOC). PMOC are generally substances of low molecular weight with a high portion of heteroatoms. They may even be ionic at neutral pH. These properties make PMOC extremely challenging to analyze, because they hamper enrichment from water as well as chromatography. Consequently, knowledge on the occurrence of PMOC in aquatic compartments (including source waters used for drinking water production) is very limited. It is thus questionable whether sufficient protection of drinking water resources with respect to PMOC is in place. PROMOTE (PROtecting water resources from MObile TracE chemicals) is a recently launched research project under the European Union Joint Programming Initiative "Water Challenges for a Changing World" (Water JPI). PROMOTE focuses on PMOC in environmental water cycles and in drinking water production. The objectives are to close the significant knowledge gaps with respect to (a) identification and prioritization of the PMOC of highest concern, (b) trace analytical methods for screening and quantitative determination of PMOC in water, (c) occurrence and levels of PMOC in groundwater and surface water, (d) environmental emissions and (e) clean-up strategies or transformation of PMOC in the drinking water production. Based on the expected results, PROMOTE strives to develop recommendations with respect to chemical regulation (REACH) and water quality monitoring (WFD watch list).

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Ranking REACH registered chemicals for Persistency and Mobility: Neutral, Ionizable and Ionic Compounds
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Herein we present a novel set of tools to identify which of the REACH registered compounds are most likely to be persistent, mobile organic compounds (PMOCs), and thereby capable of rapid and sustained distrubution in the environment and drinking water supplies. The REACH list was chosen not only because it provides an overview of which chemicals are currenlty in production, but their dossiers can contain experimental data related to mobility and persistency. We therefore conducted a literature search of these REACH dossiers as well as the peer-reviewed literature, and employed a sweet of quantitativ structure-activity relationships (QSARs) to assemble persisitency and mobility parameters. As many ionic compounds could not be handled by commercial QSARs, and only few had experimental data available, we developed simple QSARs that gave broad estimations on the likelyhood of persistency and mobility. Further, hydrolysis products were also assessed for the likelyhood of being a PMOC. These data and tools can be used to identify previously undetected drinking water contaminants, or for preventing them from occuring.

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Screening of polar chemicals in water by liquid chromatography-high resolution mass spectrometry employing mixed-mode columns
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The popularization of liquid chromatography coupled to high resolution mass spectrometry (LC-HRMS) has permitted the exploitation of its high mass accuracy and resolution capacities for screening of a large set of organic pollutants without the need of having pure standards and chemical-classes targeted methods. To date, most LC-HRMS screening methods rely on reversed-phase LC (RPLC), which is quite limited for the detection of very polar chemicals. Hence, many very polar (organic) chemicals may have not been recognized as water pollutants of concern, yet, because the analytical methods developed so far are unable to detect them. However, these polar pollutants are highly mobile in the water cycle and can spread and even reach drinking water, if they are persistent, i.e. "persistent mobile organic pollutants" (PMOC). In this context, the research project PROMOTE (http://www.promote-water.eu/), funded by the Water JPI, aims to improve the knowledge on these PMOC in the water cycle, including improving analytical methods. Thus, the goal of this study was to improve the analytical detectability of PMOC by using mixed-mode LC (MMLC) combined to HRMS, for screening purposes. MMLC provides the combination of RP and ion-exchange functionalities, which allows the simultaneous determination of analytes with extremely different properties (i.e. ionic, basic, acidic and neutral), so it is a promising technology for analyzing very polar chemicals. To reach this objective, a group of over 40 very polar model chemicals with different acid/base functionalities was employed for exploring the retention behavior and then a suspect screening approach was employed for detecting PMOC in different water samples, with several commercial and non-commercial accurate mass libraries. Some of the detected chemicals include well-known PMOC, as acesulfame, perfluorobutanoic acid, trichloropropyl phosphate or metformin, and some newly detected PMOC. *Acknowledgements* - This work is financed by MINECO (JPIW2013-117), in the frame of the collaborative international consortium (WATERJPI2013-PROMOTE) of the Water Challenges for a Changing World Joint Programming Initiative (Water JPI) Pilot Call. We also acknowledge the support of *Xunta de Galicia* ("Consolidación" funds) and FEDER.

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Identification of persistent and mobile contaminants impacting raw and drinking waters
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Highly polar organic substances may be able to penetrate natural and artificial barriers and are thus mobile in the water cycle. If these mobile organic contaminants (MOC) are persistent (PMOC) against microbiological and chemical degradation, their removal during waste water treatment and drinking water purification may prove difficult. When these substances are present in high concentrations, toxic or undergo toxication, problems for the aquatic environment and human health may arise. As a consequence of the lack of established analytical methods for MOC, only limited information about their occurrence and origin is available, which severely exacerbates the sophisticated monitoring and effective regulation of PMOC and their precursors. To identify potential PMOC, and thus begin to close this gap in knowledge we analysed 20 water samples form five European countries, including WWTP effluents, surface waters, ground waters and drinking waters. Two independent pre-treatment methods were deployed to ensure a broad coverage of potential PMOC for a subsequent HILIC-HRMS non-target screening. As sample pre-treatment methods evaporation and reconstitution and an SPE method utilizing WAX, WCX and GCB materials in one cartridge were performed. Thus the pre-concentration of the samples and the solvent exchange necessary for the sensitive analysis of water samples with HILIC-HRMS were achieved. Detected substances were prioritized based on their signal intensities and frequency of detection, whereby substances detected in drinking water were given the highest priority. To identify high priority substances, their fragmentation behaviour was investigated and H/D exchange was performed to further facilitate identification. Once a tentative identification was achieved, the fragmentation pattern as well as the retention time were compared with a reference substance, if available. If the comparison of fragmentation patterns was inconclusive or the fragmentation of a substance yielded only few or very common fragments, the retention times of the analyte and the reference substance were compared on a second column with a complementary selectivity to increase the confidence level of identification.

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Pyrazole, a polar new emerging industrial contaminant
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In the Netherlands around 40% of the drinking water originates from surface water taken mainly from the river Rhine and Meuse. Therefore several early warning monitoring stations safeguard the quality of water taken in by an array of sensors, daphnia, algae, mussels and instrumental techniques like automated HPLC Diode Array Detection (DAD). The different monitoring stations employ a common best practice protocol HPLC-DAD screening, the so-called UV fingerprint screening which is performed daily. A uniform interchangeable UV-spectrum database helps to identify unknowns without the need for a standard. Known and unknown compounds are followed by using their retention time index, their UV spectrum, and internal standard equivalents. This summer a daphnia sensor and the mussel monitor were triggered in the river Meuse. A sample was measured by the UV fingerprint screening showing a large broad peak emerging with a relative short retention index (7.62) indicating a contaminant with a highly polar nature not present in the UV database. This resulted in a closedown of the water intake for the production of drinking water. The aim of this study was to identify this new emerging compound by hyphenating the HPLC-DAD to the LTQ-FT-Orbitrap-MS. Non-target screening LCMS is most often employing electro spray ionization (ESI) as an interface but the unknown compound could not be targeted by ESI and hence the switch was made to atmospheric chemical

ionisation interface (APCI). This resulted in a clear intense corresponding signal (34 fold higher in counts than HESI) in the mass spectrometer corresponding with the exact retention time on the HPLC-DAD, but no fragmentation was observed. The structural formula was found to be $C_3H_4N_2$ and two possible suspects were selected based on structure and log K_{ow} namely imidazole and pyrazole. The latter was a perfect match and through establishing a calibration curve the concentration in the alarm sample was found to be ~100μg/l. Pyrazole is widely used as an starting product for the synthesis of pharmaceuticals and pesticides and a known industrial by-product. Now that pyrazole has been identified as an industrial contaminant in surface water, the toxic properties of the substance can be elucidated in order to establish health based guideline values for (sources of) drinking water

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Threat of drinking water resources pollution in the vicinity of Novi Sad
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The available information and results about pollution of raw water used for abstraction of drinking water in Novi Sad municipality are significant for good eco-status of surface water in river Danube, risk management as wll as for protection and improvement of human health and safety. A special interest is devoted to the Danube River as an important source of drinking water in Europe and also for Novi Sad inhabitants.The main goal of the NATO project was to reduce and prevent risks related to environmental quality of surface water and related aquifers used for abstraction of drinking water. Based on the conducted three screening and two target analyses, the raw water entering water treatment plant contained approximately 100 different organic compounds. Selected compounds represent the most frequent groups that were identified, such as linear and branched alkanes, carboxylic acids, alcohols, pesticides, hormones and others. Contamination of raw water by two emerging substances 1-octanol and benzophenone which were found in almost all samples of wastewater and Danube surface water was observed. Prioritization based on occurrence and predicted toxicity data has been conducted in order to generate the list of priority substances relevant for the water-monitoring network in the city of Novi Sad. According to those results the list of 300 relevant organic and inorganic compounds was designed, which represent a potential threat for contamination of raw water used for preparation of drinking water. Obtained list served as input data for the establishment of standard operating procedure within the Novi Sad municipality and assessment of risk for relevant detected pollutants. Implementation of joint risk management plans and strategies against hazards, which might be caused by the chemical substances in surface and raw water, could serve as an example for other cities with similar drinking water production in Serbia as well as in surrounding countries. **Acknowledgement:** The research has been supported by NATO Science for Peace Project (ESP.EAP.SFP 984087) and III46009 Project.

Ecotoxicology and risk assessment of nanomaterials - Grouping and read-across

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Preliminary outcomes of the OECD Expert Meeting on 'Grouping and read-across in hazard assessment regarding specific issues for nanomaterials'
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Manufactured Nanomaterials (MNs) are being developed in many different variations, including different sizes, shapes and surface functionalizations. While this is a positive driver for economic development and innovation, it does give rise to the need for both public regulatory authorities and industry to assess the environmental, health and/or safety concerns for all MNs that access the market. Concerns associated with a MN may be different to the one(s) for a corresponding bulk material, if existing. For those MNs there is a need to generate the data to draw the hazard profile. Whenever possible, such information should be generated by means other than vertebrate animal testing. Recently the OECD published its 'Guidance on grouping of chemicals, second edition' (2014). For MNs, it was concluded that, in addition to structural similarity, a set of physicochemical properties need to be considered for grouping and read-across. However, this issue was on purpose not further developed in the OECD Guidance due to the recognition that a better understanding of the relationships between MNs' physicochemical properties and (eco)toxicological behaviour or environmental fate is necessary before establishing accepted principles for grouping of MNs and provide recommendations on how to use existing data from bulk materials or other MNs in a read-across case. Since then, progress has been made in both regulatory and scientific communities. To discuss the most recent advancements an EU-sponsored OECD Expert Meeting on 'Grouping and read-across in hazard assessment regarding specific issues for nanomaterials' is held over two days in Brussels on 13-14 April 2016. The main focus of the OECD Expert Meeting is on producing practical, concrete recommendations on grouping and read-across for MNs for the best uptake in a regulatory context. This oral presentation will provide an overview of the state-of-the-art on grouping and read-across of MNs based on the documents and information shared at the OECD Expert Meeting as well as best practices and lessons learnt from existing case-studies. In addition, preliminary findings from the discussions in break-out sessions will be reported and discussed with the aim of outlining possible ways forward. \n

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A strategy for read-across between nanoforms
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Grouping of substances and read-across are valuable approaches in regulatory frameworks to minimise costs and animal testing. Where experimental data are insufficiently available for hazard characterisation one may in some cases predict the properties of a substance based on data from structurally similar substances. Also for nanomaterials, read-across and grouping approaches may be an important means to reduce costs of information generation and addressing data gaps. Based on existing knowledge and approaches, we developed a systematic strategy to substantiate read-across for nanoforms of the same substance with a focus on compliance with the EU REACH Regulation. The strategy comprises six different steps, including (1) identification and characterisation of the nanoform(s), (2) when possible formation of initial groups of nanoforms based on physico-chemical parameters (e.g. aspect ratio, or water solubility and dissolution rate), (3) identification of available information and data gaps for each nanoform per endpoint, (4) hypothesis driven identification of source materials to read across from, (5) where necessary additional testing to substantiate the read-across, and (6) assessing the new data and remaining uncertainties to conclude on the read-across arguments. Where read-across cannot be substantiated, the strategy foresees reiterating (some of) the steps, or performing appropriate testing to fulfil the information requirement(s) in REACH (or other regulatory framework). The presented strategy points towards the availability of data on physico-chemical parameters of each nanoform as the crucial starting point to obtain a better understanding on its (environmental) behaviour, fate, toxicokinetics and toxicity. This is the cornerstone in developing a scientific, robust justification for grouping and read-across. To improve understanding, further (international) coordination and collaboration in research is advisable. Furthermore, data quality is critical, and monitoring of physico-chemical parameters during testing is therefore a key element. This also requires harmonisation and standardisation of test methods for physico-chemical, toxicokinetics and hazard endpoints.

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Grouping of nanomaterials regarding their fate and behaviour in the environment - first hypotheses and future work
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A challenge of nanomaterials in products is their optimal use and at the same times the safe handling for humans and the environment along the whole life cycle. Several studies showed the release of NM and their transport to all environmental compartments. The application of the nanomaterial based product, the NM properties and the exposure pathway will affect the behaviour and fate of the NM in the environment and as a result the exposure of the environment, worker and consumer. The fate and behaviour in the environment and their concentration are important information for a thorough risk assessment. The potential risk has currently to be assessed case by case since no tested grouping or read across is available for the large varieties of NM. This case by case approach is time and cost consuming. Therefore, a grouping of NM and read across between different materials is indispensable and one mayor target for future risk assessment. The grouping of nanomaterials can be difficult as different perspectives and research areas are involved. Within nanoGRAVUR different aspects of grouping, read across and tiered approaches for release, exposure, hazard potential and risk analysis in view of all relevant protective targets, workers, consumers and environment are explored. The aim is to develop usable and practicable approaches within the framework of (pre-)regulative testing. One of the first steps is the set-up of different criterion catalogues based on literature

data. These catalogues will comprise NM relevant parameters such as physical chemical properties, the respective potentials of exposure, including release and environmental fate, hazard (eco- and human toxicity) and risk. Based on these catalogues grouping hypotheses for NM will be derived and evaluated in a second step. The presentation will focus on the grouping hypothesis for NM in view of environmental fate and behaviour. The first concept based on literature data, about NM transformation and mobility in air, water, sediments and soils. The concept and the planned experiments for verification or falsification of the hypothesis will be presented and discussed with the scientific community. We see the input by the scientific community at this early stage as very valuable to complete this approach and to increase the acceptability of the grouping concept in the end. The results are generated in framework of the project nanoGRAVUR which is founded from the German BMBF, Grant No.: 03XP0002

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The use of NM libraries for read across estimation - Organism and gene effects in E. crypticus exposed to TiO2-Fe doped NM library
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Nearly all ecotoxicological studies with nanomaterials (NMs) are focused on testing one or a few NMs. More systematic approaches to compare effects across many NMs with different but related characters/descriptors (e.g. different aspect-ratios or surface activities) are rarely available, especially in ecotoxicology. Therefore, there is an urgent need to provide tools that allow to develop an (eco)toxicological dataset containing comprehensive coverage of NP toxicity, with the aim of extrapolating acquired information across NMs. NM libraries are an excellent tool for alternative testing and modelling. In the present study a custom-designed Fe-doped TiO2 NM library was used, covering a wide spectrum of properties. Additionally, the reference TiO2 materials from JRC were tested (NM 103-5). *Enchytraeus crypticus* (Oligochateta) was used as test species since it is both environmentally relevant (as an important soil representative oligochaete and a standard species) and also a genomic model, with a high-throughput (HTP) transcriptomic library. Effects were assessed at the organism (survival, reproduction) and gene level (differential gene expression, microarray). Gene expressions were linked to population effects by using population Effect Concentrations (e.g. ca. EC50) as exposure levels. Material-specific effects were observed. The microarray study across the 11 materials showed that the profiles of DEGs varied among the materials. Analysis of gene ontology terms showed sub-clustering of the different materials, whereas the study of affected pathways provided the basis for mechanism interpretation.

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Predicting Nanoparticle uptake into cells by easily measurable Nanoparticle properties
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The increasing number of NP products poses increasing human health risks by particle exposure. Serious concerns about toxicological and environmental effects have been raised because in several in vitro studies adverse effects have been shown in various cell lines. Unfortunately, effects are often measured after a single fixed time point based on exposure dose, neglecting uptake kinetics and time-dependent internal cellular concentration. Two fundamental biological processes can be employed by NPs to enter cells: Endocytosis by all eukaryotic, non-phagocytic cells, and phagocytosis in specialised cells. When looking at NPs, this can be considered a so called Trojan horse effect, because cells take up the particles mistaking them for nutrients. This can result in high levels of NPs inside the cell. Various researcher show the effect of different NP properties, e.g. size and charge, on uptake into the cells. Patterns are albeit uncertain due to the lack of systematic studies and lack of determination of uptake in toxicity studies, but patterns are slowly emerging. To overcome the problem that not every of the numerous NPs can be tested in the laboratory (for uptake and/or toxicity), we aim to combine empirical studies with modelling for a cost- and time-effective risk assessment (RA). We determine uptake rate constants and elimination rate constants in dependence of NP size and charge from published experimental results. We distinguish between phagocytic and non-phagocytic cells. For non-phagocytic cells there seems to be a size optimum for uptake of 50 nm NPs based on NP numbers, smaller and larger NPs are taken up to a lesser extent. This optimum might shift when results are expressed as mass of the core material of the NPs. An increase in positive or negative surface charge leads to increased NP uptake in comparison to less or uncharged NPs. Positively charged particles are taken up more extensively compared to negatively charged NPs when the absolute zeta potential is similar. Understanding NP properties that determine their transport across cell membranes will improve our understanding of their toxicity and is crucial for RA. Predictions of NP uptake will allow to prioritize NPs for testing and to develop products that are safe-by-design. Furthermore, identification and understanding of the most important NP properties that determine NP uptake into cells is the first step towards the understanding of their accumulation along the food chain.

Amphipods as models to investigate toxicology of environmental contaminants at the land-sea interface

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Application of a multiplexed quantitation of protein biomarkers in the invertebrate species Gammarus fossarum: interest to environmental monitoring
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Sub-organismal information provided by the measurement of molecular biomarkers is a relevant approach for environmental biomonitoring (e.g. OSPAR marine strategy). Changes in molecular biomarkers can be related to an exposure to chemical compounds and some of them provide early warning indicators of possible effects on the ecosystem. However, the routine use of these tools in biomonitoring is confronted with several limitations, specifically in invertebrates. The most relevant limitations consist of the lack of specific quantification methods for each species, and the fact that each biomarker measurement involves a specific method, leading to very expensive biomonitoring strategies in time, cost and biological samples. Recently, an approach called “proteogenomics” emerged as a relevant strategy for the discovery of proteins in non-model organisms. With this approach, our consortium created a database consisting of 1873 experimentally validated proteins of the amphipod crustacean *Gammarus fossarum*, a sentinel species for continental water biomonitoring. Using this protein database, the objective of the present study was to setup an innovative approach to permit fast and specific identification and simultaneous quantification of proteins of interest in this invertebrate species. We applied a mass spectrometry based multiplexed quantitation methodology (Selected Reaction Monitoring – SRM) to study 55 proteins of interest. Identification of specific proteotypic peptides, physiological monitoring of associated proteins and their interest as biomarkers in *G. fossarum* were assessed by studying their change through male and female reproductive cycles, during food privation or after exposure to contamination (model compounds in the laboratory and *in situ* caging). This novel approach for multi-marker quantification was successfully applied for ecotoxicological analysis in an invertebrate species known for its relevance in environmental monitoring. The concentrations of several biomarkers of interest were simultaneously monitored during the physiological processes and their sensitivity to toxic contamination was demonstrated, showing the relevance of this innovative analytical approach for invertebrate ecotoxicology. In addition, we applied the measurement of molecular biomarkers for environmental biomonitoring. This breakthrough methodology in ecotoxicology constitutes a valid alternative to the time-consuming, biomarker-specific strategies currently used.

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Bioconcentration and biotransformation of selected pharmaceuticals in the freshwater amphipod, Gammarus pulex
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The aquatic environment is continually exposed to contaminants via wastewater treatment plant effluents. These compounds may have adverse effects on reproduction, development and behaviour in biota.[1] A previous investigation has shown occurrence of several pharmaceuticals in the freshwater amphipod *G. pulex*.[2] However, the potential for these xenobiotics to accumulate is not well understood. Herein, the uptake and elimination kinetics of selected pharmaceuticals in *G. pulex* is presented using both liquid scintillation counting (LSC) and liquid chromatography-tandem mass spectrometry (LC-MS/MS). Gammarids were exposed to 16 pharmaceuticals covering classes of antibiotics, anticonvulsants, non-steroidal anti-inflammatories (NSAIDs), histamine H_2 receptor antagonists, beta-adrenergic agonists and beta-blockers individually for 96 h with a 48 h uptake and depuration phase, respectively. Analysis was performed using either LSC or LC-MS/MS in positive and negative electrospray ionisation modes. Uptake and elimination kinetics were determined and bioconcentration factors (BCFs) subsequently estimated. Both methods of analysis were in good agreement and overall the bioconcentration of these pharmaceuticals remained low with BCFs ranging from 12 - 212. Different methods of modelling used to estimate the kinetic BCFs showed large variation which was attributed to a decreasing trend observed in the uptake rate constant (k_1). Furthermore the LC-MS/MS method allowed the confirmatory identification and quantification of several biotransformation products resulting from exposures to propranolol, carbamazepine and diazepam. The biotransformation indicates that these organisms are capable of Phase 1 and 2 metabolism, supporting potential further use as a replacement of fish models. Finally, the results suggested that the

bioconcentration potential of these pharmaceuticals is relatively low. References. 1) Daughton, C.G. & Ternes, T.A. (1999). *Environ. Health. Persp.*, 107(6), 907-938. 2) Miller, T.H., et al. (2015). *Sci. Total. Environ.,* 511: 153-160.

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Seasonal sensitivity of Gammarus pulex towards cypermethrin

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One of the most commonly used classes of pesticides in Danish agriculture is the synthetic pyrethroids which constitute approximately 90 percent of the total insecticide usage. The pyrethroids can enter surface waters by spray drift, colloid facilitated transport or leaching through tile drains and even short pulses of pyrethroids have been shown to be highly toxic to different aquatic invertebrates with EC_{50}-values in the ng L^{-1} range. The purpose of the current study was to investigate the variance in seasonal sensitivity of the freshwater invertebrate *Gammarus pulex* towards a short (90 minutes) pulse of the pyrethroid insecticide cypermethrin. We hypothesise that the gammarids are most sensitive during spring and the least sensitive during fall due to the general increase in fitness during summer and fall such as formation of fatty acids and proteins. To test the hypothesis, gammarids were collected in a local stream, acclimated for three days and subsequently exposed to a 90 minute pulse of cypermethrin before being transferred to clean medium for recovery, where immobilization and death were followed for seven days. To get an indication of the fitness levels of the exposed gammarids the composition of fatty acids, protein content and *in vitro* cytochrome P450 (ECOD) activity were measured in non-exposed gammarids collected at the same dates as the exposed gammarids. The results of the preliminary test period of February through October seem to support our hypothesis as the estimated EC_{50}-values were lowest during spring and early summer with the exception of March and May. The seven days EC_{50}-estimates varied from 0.29 ± 0.06 to 1.94 ± 0.60 $\mu g\ L^{-1}$ with the lowest estimate observed in April and the highest in October, respectively. This several fold difference in pyrethroid sensitivity may be explained by different fitness levels of the collected gammarids. Fitness levels measured as their lipid and protein content, are expected to increase during fall due to the increase in food sources caused by the leaf-fall. An increased protein level may very well result in a higher level of cytochrome P450 activity, which potentially can explain the decrease in sensitivity during fall, whereas an increase in lipid content might increase the proportion of cypermethrin being contained in an inactive form in the lipid fraction.

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Silver absorption kinetics in hemolymph of the marine amphipod (Parhyale hawaiensis) exposed via water

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Nowadays, silver's release into the environment is becoming an environmental concern, due, specially, to its large incorporation in nanomaterials. Absorption kinetics studies can be helpful to elucidate the toxicity mechanism of those contaminants. Therefore, the determination of silver concentration in the hemolymph of exposed organisms can be an interesting exposure measurement but, because of the relatively small size of these organisms, this task is quite challenging. The aim of this study was to evaluate the silver absorption kinetics in organism exposed to silver nitrate via water. As a next step, organisms exposed to silver nanoparticles will be studied. Experiments were performed using adult organisms of *Parhyale hawaiensis* (8 months) individually exposed. The Ag concentrations of exposure were 0; 5; 10; 25; 50 and 100 $\mu g\ L^{-1}$ prepared with $AgNO_3$ and reconstituted saline water (salinity 30). The exposure times were: 14, 24, 48, 72 and 96 hours. After exposure, hemolymph (approximately 0.5 μL per organism) was collected and weighted. Three pooled samples of 4 organisms were tested per exposure concentration and per time. The pooled hemolymph was diluted in 1 mL of HNO_3 0.05%. Copper concentration in the hemolymph was also determined and used as a possible internal standard because this metal can be found in hemocyanin. An Agilent 7700x Inductively Coupled Plasma Mass Spectroscopy was used for Ag and Cu determinations. The limits of detection for Ag and Cu were 0.043 and 0.027 $\mu g\ L^{-1}$, respectively. Silver concentrations in the hemolymph increased with the increase of Ag in water and with higher exposure times, reaching 14 ng mg^{-1}. Silver seems to be regulated by either Ag uptake inhibition or actively excretion in those organisms, especially when the ion concentration is higher than 50 $\mu g\ L^{-1}$ in the water, regardless the time of exposure. Copper concentrations remained from 60 to 120 ng mg^{-1}, regardless of variation in silver concentration in the water. The observed variation is probably due to the physiological state of the organisms such as molting stages, but additional studies are required to verify the adequacy of this measurement as an internal standard. The measurement of metals in hemolymph of such small organisms can be an interesting tool and it will be applied in silver nanoparticles toxicity studies.

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Effects of low dose radiation on reproduction and behaviour of the amphipod, Echinogammarus marinus

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Impacts of environmentally relevant doses of ionising radiation on aquatic biota are poorly understood. Significant data gaps for a range of organisms coupled with high profile nuclear incidents such as Chernobyl and Fukushima have driven a re-examination of the impacts of radionuclides. *Echinogammarus marinus* is a widespread intertidal amphipod which has gained prominence as a model species in both ecological and ecotoxicological studies. The aims of the present study were: 1) to develop a method for ecotoxicity testing of contaminants on the reproduction of model amphipods;2) To investigate the impact of chronic, low-dose ionising radiation exposure on the quantity and quality of spermatozoa in *E.marinus*;3) To elucidate the impact of radionuclides on crustacean behaviour patterns. *E. marinus* were exposed to beta radiation (phosphorous 32; P32) at a range of activity concentrations from 0 to 69 Bq/mL. Mean dose rates over the 14 day exposure period of 0.2, 1 and 9 mGy/d^{-1} were calculated. Subsequently, behavioural assays were performed using video tracking software (Ethovision XT) over an 8 minute alternating light/dark cycle. Sperm quantity and quality were ascertained using dissection and fluorescent (LIVE/DEAD) staining techniques. Following light stimulation, a significant reduction in swimming velocity was observed during dark phases of the assay at all dose rates. Exposure to P32 resulted in a decrease in sperm viability across all dose rates compared to the control although this was only significant in the 1 mGy/d^{-1} exposure group (~11% reduction). No significant differences in total spermatozoa numbers were recorded in any of the treatments. This study has developed sensitive biomarkers highlighting possible impacts of sperm quality but not quantity. A number of studies have suggested that a reduction in sperm quality may be detrimental to crustacean species at higher levels of organisation therefore ongoing research will aim to determine the presence of DNA damage and the knock-on effects on fertilisation success. These preliminary findings indicate that ionising radiation has the potential to alter male fertility and behavioural patterns at dose rates below those encountered in radioactively contaminated environments. Such studies will improve our understanding of the impacts of large scale nuclear incidents such as Chernobyl and Fukushima.

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Discussion

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Recent Developments and Current Issues in Bioaccumulation Assessment

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First insight into bioconcentration of Ionic Liquids - an in vitro approach

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Thousands of different Ionic Liquids (ILs) have been synthesized and few hundreds are commercially available. ILs are used nowadays from small-scale through pilot plant to large-scale industrial applications. This comes with the increased probability that they will be continuously released into the biosphere, for example, as process effluents or consumer products, or in larger amounts as accidental spills. Hence they should be considered as potential pollutants (PPs). Bioaccumulation is of the highest concern for environmental risk assessment of chemicals, since it is known to cause far-reaching hazards to wildlife and human health. Generally, the experimental measurement of bioaccumulation is time-consuming, expensive, and due to ethical concerns regarding animal welfare not feasible for large sets of chemicals. Thus prediction models - mainly based on easily determinable physicochemical properties such as the octanol-water partition coefficient - are usually used. However the existing prediction models often give inappropriate and inaccurate results for ionogenic compounds and permanently charged organic chemicals. This is due to the fact that classical bioaccumulation models neither sufficiently consider ion–macromolecule interactions nor interactions of cations and anions in solution - both strongly influencing the transport, uptake and bioavailability of ions. Therefore the main aim of this study was to understand the interactions of organic ions, and ion pairs in particular, with *in vitro* biological systems and their consequences in terms of bioconcentration. On the basis of obtained results strong evidence for the influence of alkyl chain length and thus hydrophobicity was presented. Moreover the influence of hydrophobic counterion on lipid membrane partitioning was determined. The study also showed that lipid membrane partitioning of ILs is concentration – dependent. Hydrophobic ILs had rather high affinity to lipid membranes suggesting significant bioconcentration potential. Since hydrophobic counterion clearly exhibited membrane partitioning of IL cation, influence of co-contaminants on bioconcentration potential should be always considered. *Acknowledgement This research has been supported by the Institutional Strategy of the University of Bremen, funded by the German Excellence Initiative.*

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Improving fish bioaccumulation assessment for ionogenic compounds by in vitro measurements of the liver S9 clearance rates
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Under the chemical legislation REACH, adequate estimates of the bioaccumulation potential of thousands of chemicals, of which 50 % is ionogenic, are required. Only recently, a bioconcentration model specifically for ionogenic organic chemicals (BIONIC) in fish has been developed. Bioaccumulation data on ionogenic organic chemicals (IOCs) are scarce, but also the QSARs used to parameterize the critical uptake rate constant, tissue distribution and biotransformation rates are poorly validated for IOCs. The available *in vivo* fish biotransformation rates for IOCs do not cover many fully ionized structures, and also do not equally cover all different types of acids (phenolates, carboxylates, sulfonates, sulfates) and types of bases (primary, secondary, and tertiary amines, and quaternary ammonium salts). We used a straightforward *in vitro* biotransformation assay with a single batch of S9 Rainbow trout liver homogenate (Rt-S9) in order to derive consistent measurements of intrinsic hepatic clearance rates ($CL_{in\ vitro,intr}$)on 50 IOCs, covering nearly all monoprotic IOC types. Since the Rt-S9 is well characterized and standardized and includes both phase I and phase II enzymatic conversions, this data set covers the baseline ability of uninduced fish to biotransform xenobiotic ionic compounds in an environmental exposure, which would be a valuable conservative approach. The next challenge is to extrapolate these values to whole organism transformation rates, after which they can be applied to the BIONIC model. The 50 IOCs include many simple chemical structures that allow for a careful evaluation of the influence on $CL_{in\ vitro,intr}$ of (A) the ionizable group, (B) the hydrophobicity (non-polar moieties) of IOC structures, (C) the position of the charged group in the IOC (interior or exterior of the structure), (D) branching (related to C), and (E) to some extent specific polar functional groups. This data set on IOCs pose an important shortcut to current uncertainties in deriving biotransformation rates from in vivo measurements for IOCs, and could present a framework to establish a more extensive Rt-S9 QSAR that specifically addresses all kinds of ionizable contaminants. The *in vitro* Rt-S9 data can further be used to evaluate and support the BIONIC bioaccumulation model k_{MET}-QSARs that are based on *in vivo* data.

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Identifying obstacles to the application of the one concentration approach for fish bioconcentration testing
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Fish bioconcentration studies assist in determining the potential for substances to bioaccumulate. The resulting bioconcentration factor (BCF) value is used as part of Persistence, Bioaccumulation and Toxicity (PBT) and secondary poisoning assessments. International data requirements for general chemicals and plant protection products (PPPs) include triggers for determining BCF values, generally where bioconcentration might be expected. Bioconcentration tests are time consuming, expensive, and use a minimum of 108 animals per study. Alternative methods that replace, reduce or refine the use of fish for BCF testing would therefore be of value in improving efficiency, reducing costs and supporting animal welfare considerations. The 2012 revision of the OECD Test Guideline for BCF testing (TG 305) provides the option to use only one exposure concentration, when justification is provided, although two exposure concentrations may still be required for some regulatory purposes. Recently this justification has been demonstrated for general chemicals and PPPs, where it was shown that one concentration can be tested without compromising the BCF determination. This provides a strong data-driven rationale for using the one test concentration approach for BCF assessment of general chemicals and PPP active substances, and where applied would reduce the number of fish used for this purpose by one third. In 2015 we conducted a survey across academia, regulatory bodies and industry to determine how often the one concentration approach is being applied in practice, and identify the barriers to its widespread uptake. Fifty-eight participants from Asia (n=3), Europe (n=21), North America (n=30), and Latin America (n=4) completed the survey. Of these, 31 respondents were scientists from organisations which conduct fish bioconcentration testing for regulatory purposes, of which 29 responded to the question 'How often does your company apply the one concentration approach in practice?'. The majority of these scientists (48%) occasionally apply the approach, with 41% never utilising the approach, and only 10% using it routinely. Twenty-two of these scientists identified barriers to its uptake. These were cited as a lack of global regulatory acceptance (64%), followed by scientific concerns (32%), and inertia (4%). In this presentation we will explore the barriers in more detail and discuss potential strategies to enable greater use of the one concentration approach in practice.

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Study of biomagnification of poly- and perfluoroalkyl substances on fresh water food webs
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Poly- and perfluoroalkyl substances (PFAS) are surfactants that have been synthetized and used in a wide variety of applications since the 1950s. As a consequence, PFAS are introduced via multiple pathways in the environment and some of these compounds have been detected in all compartments including aquatic systems. Even if PFAS concentrations are rarely superior to µg/L in surface waters, elevated concentrations have been reported in aquatic organisms (up to mg/kg) demonstrating their bioaccumulative properties. Recent works highlighted the importance of the use of metrics such as the Trophic Magnification Factor (describing the behavior of a compound along a food web) for bioaccumulative assessment of persistent organic pollutants. In this study, the biomagnification potential (TMF) of 23 PFAS was investigated in freshwater riverine food webs, for the first time. Thus, various biota species including fish (barbels *Barbus barbus* and chubs *Squalius cephalus*) and invertebrates representing different trophic levels (primary consumers, omnivores, herbivores, carnivores or filter feeders) were collected across 4 sites in the Rhône basin and 1 site in the Loire Basin (Eastern France). Analysis of stable isotopic ratios ($\delta^{15}N$ and $\delta^{13}C$) allowed for the determination of trophic levels (TL). Several regression approaches were compared for TMF calculation, including a classical regression, and regressions taking into account sample heterogeneity and/or left-censored values. At each site, particular care was devoted to the choice of the organism at the baseline of the food web. Fish were consistently at the top of the food chain (TL = 3-4.5) and were significantly more contaminated than invertebrates (ΣPFAS = 6–2321 *Vs* 0.8–205 $ng.g^{-1}$ ww). The most frequently detected PFAS were long-chain carboxylates and sulfonates, while shorter chain PFAS were never detected. TMFs ranged between 1.5 and 6 for long-chain PFAS, strongly suggesting their biomagnification potential in the riverine food webs investigated. TMFs could vary substantially among sites, and according to the regression model used.

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Measuring Trophic Magnification Factors: Role of Spatial Concentration Gradients, Disequilibria and Field Sampling Design
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Trophic magnification factors (TMFs) are field based measurements of the bioaccumulation behavior of chemicals in food-webs. TMFs can provide valuable insights into the bioaccumulation behavior of chemicals. However, like other bioaccumulation metrics, TMFs are subject to considerable uncertainty and the influence of numerous confounding variables. This study seeks to investigate the role of spatially-variable concentrations in water and sediments on the determination of the TMF. For this purpose, a multi-box food-web bioaccumulation model was developed to account for horizontal and vertical spatial concentration gradients and species movement on chemical concentrations in aquatic biota of food-webs and TMFs. Model testing through a comparison of model calculated and observed TMFs for persistent PCB congeners and biotransformable phthalate esters in a marine aquatic food-web (subject to spatial variations in chemical concentrations in water and sediments) showed good agreement between model calculated and observed TMFs. Model testing showed no systematic bias and good precision in the estimation of the TMF for PCB congeners but an apparent underestimation of the TMFs for phthalate esters. A model sensitivity analysis showed that species sampling designs that ignore the presence of concentration gradients can misidentify the TMF. The determination of the TMF is most sensitive to concentration gradients and species migration patterns for substances that are subject to a low degree of biomagnification or trophic dilution (i.e., TMFs around 1). TMFs were found to follow a strong relationship with log KOW and to be sensitive to biotransformation rates. Model calculations indicate that systems with relatively homogeneous exposure are best suited to determine TMFs that are representative of chemical bioaccumulative properties. The model is useful in anticipating the effect of spatial concentration gradients on the determination of the TMF; guiding species collection strategies in TMF studies; and interpreting the results of field bioaccumulation studies in study locations that are subject to spatial differences in chemical concentration.

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A bioaccumulation assessment using a weight of evidence approach and Triclosan as an example
S. Pawlowski, BASF SE; J. Arnot, ARC Arnot Research & Consulting Inc.; S. Champ, BASF SE
The assessment of the bioaccumulation potential of a substance according to current regulation requirements such as REACh is based on the logKow (screening) for the first instance, followed by a fish bioconcentration factor (BCF) study following OECD 305 test guidelines for the final assessment. The

standardized fish BCF study results are usually considered definitive for the "B" assessment and the potential for accumulation along the food chain (secondary poisoning). Normalized to a 5% lipid content and corrected for growth, this value is considered as representative for aquatic species (i.e. fish). However, in some cases where BCF values from several tests are avialable, these values can be highly variable indicating an uncertainty among those data. For example, in case of Triclosan, an antimicrobial active substance, at least three guideline fish BCF studies are available revealing BCF values from about 100 to about 8700 L/kg. Other "B" metrics such as biomagnification factors (BMFs) and trophic magnification factors (TMFs) and toxicokinetic information such as the total elimination half-life (t1/2) can also be considered as lines of evidence in a weight of evidence approach for "B" assessment to address uncertainty in measured BCFs and to more fully characterize the bioaccumulative properties of a chemical. Taking all of these lines of evidence into account, it can be concluded that Triclosan does not exceed the commonly used BCF bioaccumulation criteria (thresholds). Furthermore, models parameterized with the available data calculate biomagnification factors that are less than 1; Triclosan does not biomagnify in food chains.

Environmental risk assessment of chemical mixtures: the steps ahead (II)

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Mixture toxicity assessment using tissue concentrations - experiences from analysing real-world monitoring data
T. Backhaus, University of Gothenburg / Department of Biological and Environmental Sciences; D. Yngsell, University of Gothenburg / Department of Biological and Environmental Science; M. Gustavsson, University of Gothenburg
Norway has been monitoring populations of cod and trout since 1998 for the presence of organic contaminants including classic POPs from the "dirty dozen" such as PCBs, polychlorinated dibenzo-p-dioxins and -dibenzofurans, but also more "modern" pollutants such as flame retardants, alkylphenols, triclosan and perfluorinated compounds. Aim of the presented study was to assess whether the joint body burden of Norwegian cod and trout populations indicate an impairment of fish population health. Two sets of monitoring data were made available by the Norwegian Environment Agency for this purpose: (i) data on the pollution burden of trout populations monitored between 2010 and 2014, (ii) data collected between 1998-2013 for cod populations. In total the body burden of 151 organic compounds was monitored.\nFor each compound estimates were collected on bioaccumulation (BAF) and chronic toxicity to fish (QSAR estimates, experimental data, PNEC and EQS values). These were then combined with the biomonitoring data in order to estimate the overall risk of the exposed fish populations using Concentration Addition. For this purpose all aquatic concentrations were first re-calculated to internal body burdens by multiplying each aqueous concentration with the corresponding bioaccumulation factor. Results indicate that final risk quotients are well below one for the majority of cases, although the analysis pipeline is fraught with substantial uncertainties. In particular (i) there is a paucity of reliable toxicity data for a majority of the compounds, (ii) the application domain of existing QSAR and bioaccumulation models is too restricted to encompass highly lipophilic compounds such as e.g. several of the brominated flame retardants that were commonly found pollutants.\n\n

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Hierarchical Bayesian approach to reduce uncertainty in the aquatic effect assessment of realistic chemical mixtures
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Species in the aquatic environment differ in their toxicological sensitivity to the various chemicals they encounter. In aquatic risk assessment, this interspecies variation is often quantified via species sensitivity distributions. Because the information available for the characterization of these distributions is typically limited, optimal use of information is essential to reduce uncertainty involved in the assessment. Here, we show that the credibility intervals on the estimated potentially affected fraction of species after exposure to a mixture of chemicals at environmentally relevant surface water concentrations can be extremely wide if a classical approach is followed, in which each chemical in the mixture is considered in isolation. As an alternative, we propose a hierarchical Bayesian approach, in which knowledge on the toxicity of chemicals other than those assessed is incorporated. A case study with a mixture of 13 pharmaceuticals demonstrates that this hierarchical approach results in more realistic estimations of the potentially affected fraction, as a result of reduced uncertainty in species sensitivity distributions for data-poor chemicals.

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Evaluation of pesticides monitoring programmes using a process based mixture model
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A number of European countries run large scale pesticide monitoring schemes aimed at identifying and evaluating the presence of pesticides in the environment. We compared data from a regional monitoring programme in the UK with the country wide monitoring programme in the Netherlands. In total over 15,000 samples containing over 1 million individual measurements were evaluated for effects, using a process-based mixture model that relates observed concentrations to effects on the survival probability of *Daphnia magna*. The monitoring data were analysed for effects, including and excluding below detection limit results. It showed that there are only a small number of places where we can expect to have effects on daphnids, based on measured concentrations. However, the most polluted samples would cause extinction of a daphnid population within only 30 hrs. If below detection limit measurements are included in the analysis (like the Water Framework Directive prescribes), in up to 35% of the Dutch samples the effect of the simultaneous exposure to all pesticides is such that a direct threat to the survival of daphnids exists. Our analysis also showed that detection limits in the monitoring programmes are basically too high to exclude additive mixture effects and so to make a statement on whether or not the environment is actually protected. To improve on this predictive mixture modelling can be used in combination with a more focussed monitoring programme.

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Thresholds for synergy: How to define at what concentration a synergist stops acting as a synergist
N. Cedergreen, University of Copenhagen / Plant and Environmental Sciences; E. Becerra, D.S. Paludan, K. Dalhoff, M.A. Bjergager, University of Copenhagen / Department of Plant and Environmental Sciences
Synergy has long been a topic of concern in cummulative risk assessment. If toxicants act as synergists, they might enhance the effect of other chemicals beyond what is predicted with mixture models, which assume no interactions between chemicals. Tests for synergistic interactions are, however, most often performed with concentrations of the synergists being much higher than the concentrations occuring under natural conditions. It is likely that there is a lower threshold concentration below which the action of the synergist is neglible. The aim of this study was therefore to explore different ways of determining lower thresholds for synergistic interactions, using the known synergistic fungicides prochloraz and propiconazole together with the pyrethroid insecticide alpha-cypermethrin. Different setups and statistical methodologies were tested and lower thresholds for synergy on survival was determined for the two azole fungicides in combination with alpha-cypermethrin for the aquatic macroinvertebrates *Daphnia magna, Chironomous riparius, Gammerus Pulex and Hyallella azteca*. Synergy was defined as happening in mixtures where either EC_{50} values decreased more than two-fold below what was predicted by concentration addition (horizontal assessment) or as mixtures where the frequency of immobile animals increased more than two-fold above what was predicted by independent action (vertical assessment). Finally, survival over time was evaluated using a GUTS TKTD model letting the elimination rate constant, k_e, depend on the azole concentration. Horizontally assessed thresholds were 1.2-4.7-fold lower than vertically assessed thresholds for *D. magna*. By evaluating the effect of time, the vertical threshold was found to decrease with increasing test duration from 0.026±0.013 μM and 0.425±0.089 μM for prochloraz and propiconazole in the 48h standard tests, to 0.015±0.004 μM and 0.145±0.025 μM, in the 14-days tests on *D. magna*. The threshold concentrations for the three other species were within an order of magnitude of the *D. magna* thresholds. The GUTS modelling showed a strong decrease of k_e with increasing azole concentration, indicating that effects on k_e might also be used for setting thresholds for synergy.

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Combined toxicity considerations in a constituents-based environmental risk assessment of multi-metallic substances
K. Oorts, F. Iaccino, ARCHE; V. VEROUGSTRAETE, Eurometaux / EHS; H. Waeterschoot, Eurometaux
There is a clear need to include a generic approach to address combined toxicity when assessing multi-metallic substances such as inorganic UVCBs, for which the assumption was made that the toxicity is driven by the toxicity of their constituents, resulting in parallel risk assessments of the constituents. Upon ECHA's request REACH registrants of inorganic UVCB intermediates committed to improve the combined toxicity assessment in their registration dossiers. However, an appropriate standard approach to address combined toxicity of metals in a regulatory framework is still missing: most standard approaches yield indeed over-conservative results such as risk scenarios at background concentrations when several metals are combined. Numerous research efforts have been performed during the last years in the field of combined toxicity of metals in the environment. This research is usually focussing on high effect concentrations, specific combinations of up to maximally 4 metals and on few standard test organisms, and restricted to well studied metals, such as Cu, Zn, Cd and Ni, with well characterised ecotoxicological effects to organisms in the water, sediment

and soil compartments. However, multi-metallic substances like the inorganic UVCBs can contain more than 10 constituents, including 'data poor' metals such as As, Pd and Te. No clear conclusions have yet been formulated on a generic approach, i.e. extrapolated to any combination of metals and to all organisms within an environmental compartment. A one day workshop was therefore organised in October 2015 with participants from academia, regulatory bodies and industry. The aim of this workshop was to bridge the gap between the scientific developments and current regulatory requirements on combined toxicity of metals. This workshop resulted in a proposal for a generic tiered approach, starting from the standard concentration addition evaluation based on summation of the PEC/PNEC ratios of the individual constituents. Several options for refinements of this standard approach, e.g. taking into account bioavailability, using msPAF calculations or assessment per trophic levels, are proposed in higher tiers, as well as screening approaches for evaluating the relative importance of the data poor constituents. This tiered approach is being tested for some case studies. In addition, several open research questions were identified for future improvement of the combined toxicity assessment of metals.

Expanding LCA: looking at organizations and at new policies

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The EU Organisation Environmental Footprint Sector Rules (OEFSR) for the retailer sector
S. Humbert, Quantis
The European Commission started a pilot to create a "Single Market for Green Products", that aims at facilitating better information on the environmental performance of products and organisations. 26 pilots made of companies, industrial and stakeholder organisations are drafting respectively 24 Product Environmental Footprint Category Rules (PEFCR) and 2 Organisation Environmental Footprint Sector Rules (OEFSR). One of the pilot is drafting the OEFSR for the retail sector and is composed by retailers (Carrefour, Colruy, Decathlon, Picard, Kering, Office Depot), public agencies (EAA from Austria, ADEME from France and ENEA from Italy), one NGO (Global 2000), one association (PERIFEM) and one LCA consultant (Quantis). As of November 2015 an assessment of the impacts of an average retailer has been performed using the 15 impact categories required. A first OEFSR has been drafted, submitted for public consultation and accepted by the Steering Committee. The results of the assessment, the draft and its main methodological points (e.g., for direct, as well as upstream and downstream indirect contributions), as well as the benefits of this OEFSR for companies will be presented highlighting latest developments and feedback, including from the supporting studies. These points also include the issue pertaining to consistency with the product approach for a sector as interdisciplinary as the retail sector. As an example of results, an average general retailer supplying products for 3'000'000 people can have a carbon footprint in the order of magnitude of 10'000'000 t CO2-eq per year, most of it being associated with the life cycle of its products sold. Interaction between OEFSRs and PEFCRs such as cross cutting issues and consistency will also be addressed. As an example of methodological agreement that has been reached among sectors is how allocation among meat, milk, pet food and leather should be performed among cattle co-products. Such type of agreement is key for a sector like the retail to be able to consistently perform its Environmental Footprint. One of the significant differences with traditional corporate footprint is that assessment and reporting for OEF goes beyond the traditional carbon footprint and includes impact categories such as water footprint, pressure on resources as well as impact on human health through environmental pollution. Pressure on biodiversity or deforestation throughout the supply chain is also included.

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Arla Foods Environmental Profit and Loss Account (E P&L) - Organisational LCA with Monetarisation
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Arla Foods is among the world's largest dairy companies. To document the total life cycle environmental impact of their product portfolio, Arla Foods has conducted an Environmental Profit and Loss Account (E P&L). The E P&L expresses Arla Foods' environmental impacts in monetary units, in addition to the underlying physical units. The functional unit of the study is Arla Foods' product portfolio in 2014. Arla Foods intends to using the results to evaluate their environmental strategy and in various communications. This paper discusses the results and learnings from the E P&L. Especially, the similarities and differences with product LCA as well as the added value of monetarising the impacts are discussed. An E P&L can be described as a means of placing a monetary value on the environmental impacts along the entire supply chain of a given business. The only difference between an organisational LCA (OLCA) and an E P&L is that the E P&L uses monetarisation as weighting in the life cycle impact assessment, which is commonly not done in LCAs and OLCA. The study includes all Arla foods' sites (99 sites in 12 countries). Production and use of raw materials, energy carriers, packaging and transport are included, as well as treatment and utilization of by-products and wastes. In addition, products and services not directly used in production, such as computers, furniture and travelling are covered. The downstream parts of the life cycles (retail and consumers) are also included. The study presents results calculated using both consequential and attributional modelling in the life cycle inventory. Indirect land use changes are included. The monetarised impacts, i.e. the investigated externalities, can be compared to Arla Foods revenue at 10,600 million EUR2014, which indicate the created value. When monetarising the impacts, the consequential and attributional approaches show a contribution at 1840-5850 and 2240-4980 million EUR respectively. The intervals represent different valuation methods. The consequential results are generally higher than the attributional. This is because they include indirect land use changes and thereby a significant impact on nature occupation (biodiversity). The E P&L gives a broad and deep insight in the impacts from the full life cycle of Arla Foods product portfolio, and it provides a good basis for comprehensive sustainability reporting and for identifying options for improving the performance and reducing the impact.

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Does ex ante application undermine the usefulness of LCA?
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Introduction\nIncentives for exploring options for safe recovery of metals from WEEE include:\n• Increasing development driven demand for metals\n• Wastefulness of metal loss into the environment\n• Deleterious environmental effects of unsafe disposal\nBioleaching, a natural process mediated by micro organisms, may become a promising emerging technology contributing to safe secondary metal recovery from WEEE.\nInitial experimental bioleaching results on WEEE printed circuit boards show efficient metal recovery yields.\n\nMethod\nLCA methodology was applied at an early stage to the novel bioleaching process to embed it in a life cycle context, linking it to upstream and downstream flows.\nA short term future scaling up scenario was defined using a proxy technology and estimated data.\nEnvironmental hotspots of this scenario were identified and its environmental impacts were compared with those of a current industrial pyrometallurgical technique.\n\nResults\nPotential hotspots were related to energy and material inputs for the bioleaching process and solvents for copper recovery.\nComparison with an existing technology returned an inferior environmental performance, even after further optimisation.\nThese results could not be considered robust given the precociousness of application, yet they served as valuable preliminary information.\nThe uncertainties also prompted further enquiry about the chosen product system boundary and the comparability of the technologies.\n\nConclusion\nThe ex ante application of life cycle assessment on an emerging technology created new knowledge on its potential development.\nApplying ex ante LCA and an exploratory scenario brings a systematic rigour and discipline to an ambiguous situation.\nThough imprecise with much conjecture involved, it is a valid mock-up of a plausible future providing useful provisional insights to be built upon.\n• Despite uncertainties, LCA displays potential environmental hotspots. \n• The developmental challenges for the emergent technology gain definition at an early stage. \n• The LCA approach broadens the research scope, bringing a systems approach, long term view, environmental aspects, and alternative perspectives on the novel technology to the research domain. \n• Ex ante LCA + exploratory scenario is of great service as a developmental design tool.

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Developing life cycle sustainability indicators for setting up a new carbon fiber recycling sector
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Carbon fiber reinforced plastic (CFRP) consumption has been increasing over the past decade in sectors such as aeronautic, aerospace, automotive or wind energy. The trend also predicted to increase in the future, inevitably there will be a large amount of waste from the CFRP manufacturing process and its use. This urges for the need to establish and implement a sustainable recycling sector focusing on carbon fibers. With this context, the SEARRCH (Engineering Sustainability Assessment Research for Composites with High Recycled value) project aims at defining a list of Key Sustainability Performance Indicators (KSPi) inherent to the CFRP recycling industry in order to help stakeholders make their choices easily. As research partner of SEARRCH project, in this paper we focus on the selection of suitable indicators and assess their relevance to meaningfully define the CFRP recycling sectors' sustainability. The selection of relevant indicators was carried out based on an extensive literature review on existing methods and indicators used to assess the three aspects of sustainability. Environmental indicators were based on life cycle assessment (LCA). The frequency of use was considered as the main selection criterion from a number of indicators analysed. The socio-economic aspects of carbon fiber recycling sectors were addressed by considering an additional resource perspective, which is based on recent publication by Sonnemann et al., 2015. The results show that global warming (IPCC) acidification (Accumulated Exceedence), and human concern (USEtox) are the most relevant environmental indicators. Three indicators were also

identified to assess the socio-economic aspect of carbon fiber recycling from resource perspective. These are: supply risk due to geological availability (CML -Resource Depletion), supply risk due to geopolitical factor (GeoPolRisk) and importance (waste flows obtained from the material flow analysis (MFA)). For the economic aspects the net present value (NPV) that allows to consider stakeholders and potential investor point of view was selected. The paper highlights the possibility to bring aspects of criticality assessment as a new resource dimension that can be integrated with the traditional environmental assessment tools to address the sustainability performance of a newly created carbon fiber recycling sector. The future perspective is to apply the proposed indicators to a specific case study.

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Assessing resource efficiency of production processes in a Life Cycle approach: the case of metal working processes
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Enhancing resource efficiency is a major goal of sustainable development. Manufacturing industries are responsible for around 25 per cent of the primary resource use and a third of the global electricity use [1]. As to metalworking industries in Germany, major potentials to increase energy and resource efficiency at company have been identified in previous studies. However, the implementation of these potentials is slow. In the research project 'TU Darmstadt learning factory–resource efficiency in production–pilot project-machining processes'[1] we investigated technological parameters and indicators as basis for guidance documents for metal working companies. The focus of our research project was 1) to detect the relevant parameters of machining processes (drilling and milling) on company level, which have a high impact on resource and energy consumption and 2) to create concepts of indicators for assessing resource efficiency of machining processes on life cycle level. European resource policy defines the term of resources as materials extracted from nature but also the carrying capacity of the natural environment [2]. Given this definition, Life Cycle Assessment is a suitable methodology to assess resource consumption as well as flows to the environment connected to impact categories. However, enhancing resource efficiency in the manufacturing industry requires robust and transparent indicators of resource consumption which can be easily communicated within companies. Using the LCA as assessment methodology we found out that during the examined machining processes the consumption of electricity, compressed air, cooling lubricants and the MQL oil are relevant parameters for resource efficiency. On this basis, we derived indicators which can be used for metal working processes on the company as well as on policy level. References [1] UNEP. 2011. Manufacturing. Investing in energy and resource efficiency. http://www.unep.org/greeneconomy/Portals/88/documents/ger/GER_7_Manufacturing.pdf Accessed 23.11.2015. [2] European Comission. 2010. A strategy for smart, sustainable and inclusive growth. COM(2010) 2020.

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How to improve the valorisation process of End-Of-Life vehicles? LCA as a tool to help decision
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This paper undertakes an environmental evaluation of hybrid vehicles recycling, using industrial data from Comet Traitement SA in Belgium. Three business lines have been modelled and analysed. The first one is relative to the business as usual with a dismantling to recover batteries and engines followed by shredding and post shredding treatments. The second one considers, in addition, the removal of electronic control units (ECU) before shredding followed by same steps than in the first line and the last one is relative to the additional removal of big plastic parts before shredding and business as usual post shredding treatments. Environmental impacts are assessed using both attributional and consequential LCA methodology both ILCD 2011 midpoint and ReCiPe 2008 methods.This study tends to answer this question: "Are the recovery of plastics and ECU prior shredding environmentally relevant in the end-of-life vehicle recycling process?". Using these environmental impacts results, important steps of recycling in both alternative routes will be highlighted. This study will also allow a comparison between these routes using several environmental criteria. This information could be coupled with economic costs of this dismantling and will give clues relative to this implementation of processes in Belgium and then information for policy decisions.\n Removal of ECU and plastics before shredding show an environmental gain in all categories compared to the business as usual scenario but differences are non-significant, lower than the uncertainty of the results, excepted for ECU where it depends on the yield valorisation. Concerning plastics, additional environmental gain is always negligible. This LCA shows the limit of dismantling in terms of environmental benefits and the highlights given by this tool for policy making. \n

Ecotoxicological assessment and water quality monitoring in support of marine and freshwater legislation in Europe

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Marine contaminants and biological effects assessments for policy purposes; OSPAR's work at the regional scale
J. Foden, OSPAR; T. Burgeot, IFREMER / Department of Biogeochemistry and Ecotoxicology
This talk will provide a policy perspective, presenting OSPAR's work in assessing contaminants and biological effects in the marine environment, at the regional-scale. It will highlight how OSPAR's Contracting Parties of the North-east Atlantic maritime area cooperate to share the burden of monitoring and assessment. Data are freely available from an ICES data portal, and are used by OSPAR, HELCOM, AMAP and Expert Groups in the management of chemical and biological data for regional marine assessments. OSPAR's expert group conducts and publishes annual assessments of contaminants (http://www.ospar.org/work-areas/cross-cutting-issues/cemp). The talk will describe OSPAR's Intermediate Assessment 2017 (IA2017) and its constituents of 'common indicator' and 'thematic' assessments. The dual purpose of the IA2017 as a 'roof report' to assist OSPAR Contracting Parties that are EU Member States in their reporting commitments for the EU's Marine Strategy Framework Directive will be explained.

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SIMONI: Smart integrated monitoring of the water quality
R. van der Oost, Waternet / Onderzoek en Advies; G. Sileno, Waternet / Research and Development; L. Moria, Waternet / Water Systems; M. Thao Nguyen, Waterproef; M. Suarez Munoz, University of Amsterdam IBED Institute / Institute for Biodiversity and Ecosystem Dynamics
At the Waternet Institute for the Urban Water Cycle an alternative effect-based chemical monitoring strategy has been developed over the last five years. The objective of this SIMONI strategy (Smart Integrative Monitoring) is to get more information on the water cycle quality for less money than traditional methods (e.g. Water Framework Directive). This project, that aims to bridge the gap between scientific research and regular field monitoring, combines passive sampling with standardized *in situ*, *in vivo* and *in vitro* bioassays. The first version of the SIMONI model for classifying microchemical risks of surface waters will be demonstrated. A simple 'Toxicity traffic light' is developed for policymakers and regulators, indicating low, potential and high risks of microchemical pollution for the ecosystem. Phase 1 of the model is a hazard identification that makes the distinction between low and potential ecological risks. Hazards are mainly determined with a suite of reliable, fast, user-friendly and inexpensive bioassays. The selection of endpoints for toxicity profiling was directed towards the indication of potential risks from a broad spectrum of chemical micropollutants to aquatic organisms (e.g. nonspecific toxicity, endocrine disruption, antibiotics activity, genotoxicity, oxidative stress, dioxin-like toxicity). In order to indicate ecological risks, effect-based trigger values (EBT) were designed for all relevant bioassay responses. A three-step approach was used for EBT development, aquatic toxicity data search, species sensitivity distribution of toxic equivalents (TEQ), and benchmark field studies. Measured bioassay data were incorporated into a simple model that compares the responses with their EBTs and adds a weight factor to different types of bioassays, thus generating a quantitative hazard classification of the entire mixture of micropollutants. Results of field monitoring studies will be presented that demonstrate the feasibility of this strategy for identifying hot-spots of chemical pollution. It appeared that highest ecological risks occurred at the agricultural greenhouse areas, most likely due to pesticide emissions, while lower risks were observed in waters receiving wwtp effluents. Due to low costs and high relevance, this model has the potential to become the first bioanalytical strategy to be applied in routine water quality monitoring. *Key words: toxicity profiling, passive sampling, ecological risk assessment*

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Environmental monitoring: industry requirements and needs
I. Nilssen, Statoil ASA and Norwegian University of Science and Technology / Environmental Technology
Environmental monitoring related to the offshore petroleum industry has traditionally been carried out through ship campaigns with varying temporal and spatial resolution. Typical parameters measured are physical parameters such as grain size and total organic material (TOM), chemical parameters such as heavy metals and hydrocarbons as well as biological parameters such as taxonomy, species diversity and condition. National requirements are highly variable but the main purpose is to define the polluted area and trends within and between various areas. Linking environmental monitoring to the industry's relevant risk picture, however, traditional monitoring may not be the most cost-efficient. The reasons are several and they all originate from the need of flexibility. The activities on the different assets vary, the nature of the discharges varies and they are located in areas with various environmental conditions. In a risk-based system, monitoring, risk modelling and mitigation measures are the three cornerstones. Modelling can help optimising the environmental monitoring programme by focusing on the most critical aspects and sampling strategy. New technology has led to new opportunities for a holistic environmental monitoring approach adjusted to purpose, object or specific area of interest. A variety of fixed and mobile sensor

platforms now enables gathering of relevant amounts of data with the temporal and spatial resolution needed. Combining monitoring and modelling with appropriate mitigation measures, targeting the most critical component(s) of the discharges, will lead to improved environmental performance.

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Use of effect-based tools in the context of the Water Framework Directive (WFD) – presentation of past and on-going projects
S. Schaan, European Commission - DG Environment / Water unit
Effect-based tools are drawing increasing interest in the context of the implementation of the Water Framework Directive (WFD), in particular because of their ability to detect effects caused by mixtures of pollutants and because of their potential use to bridge the gap between a substance-by-substance risk assessment (used to assess the chemical status in the WFD) and the integrative biological indicators used to determine WFD ecological status. This presentation will describe past and on-going activities on the use of such tools led under the Common Implementation Strategy for the Water Framework Directive, and outline some future perspectives.

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Developing a framework for the assessment of contaminant impacts in marine ecosystems
K. Hylland, University of Oslo / Department of Biosciences; I. Davies; M. Gubbins, Marine Scotland; C. Robinson, Marine Scotland Science; T. Lang, Thünen Institute; D. Vethaak, DELTARES / Marine and Coastal systems; C. Martinez-Gomez, Instituto Español de Oceanografia / Marine Contamination and Biological Effects; T. Burgeot, IFREMER / Department of Biogeochemistry and Ecotoxicology; J. Thain, Cefas
European marine ecosystems are subject to a range of pressures, including fisheries, habitat change and contaminant inputs. Available data suggest that contaminants affect marine organisms, particularly linked to inputs from large rivers and areas with high offshore activity. Despite such knowledge, there has been no widely accepted guideline by which to assess contaminant effects. An integrated framework has been developed by ICES/OSPAR working groups (WKIMON/SGIMC) to enable an assessment of contaminant impacts in coastal and offshore ecosystems. The framework is based on assessment criteria for contaminant concentrations and effect methods, i.e. identifying background assessment concentrations (BACs), and environmental/ecotoxicological assessment criteria (EACs) for selected contaminants and biological effects methods. The framework comprises abiotic and biotic components, i.e. sediment, fish, mussels and gastropods. In addition, additional measurements to support the assessment, e.g. hydrographical data, are included. A methodology has been developed to combine results from different matrices/species and analytical methods, producing indices that may be integrated across methods/mechanisms of toxicity, locations or regions. The index for any single location or region using the framework can be integrated with assessments performed within the Marine Strategy Framework Directive.

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Panel discussion

Science Integrity and Publication Bias

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Introduction to Research Integrity – Academic Perspective
M. Zeegers, Maastricht University
There is a broad consensus among academic institutions, government and industry that more systematic and explicit attention should be paid to scientific integrity. Responsible Conduct of Research (RCR) is not only an ethical responsibility. It is also a relevant operational priority in times when there is increased scrutiny from government, regulatory authorities stakeholders and the public, and the reproducibility of research findings appears to be low. Previous studies, however, have shown however that on average 2% of scientists conduct fraud and 34% are involved in Questionable Research Practices (QRP), which due its high frequency may have a larger organizational impact. This whistleblowing presentation discusses the current play-field and proposes some policy changes to reduce 'research waste'.

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Research Integrity Codes – An Overview in a Governance Perspective
L. Sydnes, University of Bergen
Science is an enterprise that is based on trust and transparency. Knowledge is supposed to be generated, communicated, and distributed a verifiable and provable way that is assumed to prevent fraud and other dubious practices and make sure the science community is self-correcting [1]. Of course we know that the system has never been completely flawless, but overall it has functioned so well that most knowledge made publicly available has been reliable and trustworthy. However, the last couple of decades we have witnessed a sharp increase in the number of violations of this vulnerable and delicate system. This is alarming because the value and benefits of research as well as the sciences' ability to serve society and humankind are vitally dependent on the trustworthy researchers performing investigations with integrity [2]. To meet this crucial challenge and try to curb scientific misconduct, discussions and developments of Code of Conducts have increased considerably in recent years and made the science community aware of the need to be proactive and teach and reflect on what constitute research integrity as the framework for science changes. In the lecture cotemporary challenges and initiatives relevant to the theme will be presented, discussed and highlighted. Attention will be drawn to available resources, including ICSU's compilation of documents on the web [3], IUPAC's living-code concept [4], and publications resulting from conferences [5], which are of value for researchers and teachers alike. *References* [1] CFRS brochure; *Freedom, Responsibility and Universality of Science*, ICSU, Paris France; **2014**; http://www.icsu.org/freedom-responsibility/articles_and_letters. [2] 2nd World Conference on Research Integrity; *Singapore statement on Research Integrity*, **2010**; http://www.singaporestatement.org. [3] See http://www.icsu.org/freedom-responsibility/research-integrity/statements-codes-reports [4] G. S. Pearson, E. D. Becker, L. K. Sydnes; Why Codes of Conduct Matter, Chemistry International **2011**, *33* (6), 7-11. [5] N. Steneck, M. Anderson, S. Kleinert, T. Mayer (eds.); *Integrity in the Global Research Area*, World Scientific, Singapore; **2015**.

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Sound Science – Industry Perspective
D. van Wijk, Euro Chlor
The chemical industries and their products are essential for societal prosperity and innovation. Innovation depends on effective scientific progress, which is also a key determinant in how the assessment of chemical safety for human health and the environment is performed in order to enable evidence-based decision-making. Therefore the scientific investigation and publication process and the utilisation of scientific results are essential for industry and the entire society as a whole.\nIn a society with different interests, science has to provide the level playing-field and common ground and therefore it is essential that science integrity is maintained at all levels during science's route from concept to law. In practice though, there are vulnerabilities in the process that may occur at different application levels, in a multitude of different ways. To identify and illustrate good and bad practices at all levels from investigation to policy, a matrix approach has been developed which will be discussed.\nAs a worst-case example, the incorporation of poor studies into poorly reviewed journals that are 'cherry-picked' into review articles, may lead to selection by a prejudiced scientific committee for inclusion by legislative bodies who are motivated by popular opinion (based on tabloid/ social media 'scare-mongering'). This may result in regulations that incorrectly restrict societal use of essential substances. The presented matrix can help to identify those best practices that are required at each stage in directing impartial, apolitical and scientifically correct, high quality decision-making.

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Perspectives of Academic Journals on Scientific Integrity and Publication Bias
G. Burton, University of Michigan / School of Natural Resources   Environment
There is an increasing awareness of a lack of scientific integrity and publication bias in peer-reviewed journals. This has been documented in the highest ranked journals. Retraction rates have dramatically increased due to plagiarism or falsification of data. This increasing trend is likely due to both improved detection methods and pressures to publish. The rates of bias are less clear and more difficult to measure and are linked to sector affiliation, gender, age, institutional reputation and geography. The majority of those surveyed support the double-blind review process to reduce bias, but there is no consensus across scientific journals. Promoting publication from the disenfranchised is a noble call – but requires concerted efforts from scientists and professional scientific societies. ET&C is addressing these challenging issues via a number of avenues, including their "Perspectives" columns, routine dialogue with their Editors to identify and respond to bias, and actively recruiting submissions from under-represented regions. ET&C will continue to be vigilant in maintaining its high level of scientific integrity, and will continue to look for methods to reduce the inherent human tendency of bias. Organizing a mentoring approach for scientists in under-represented regions has been of an *ad hoc* nature and should be expanded in the future.

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SETAC Ethical Code of Conduct - followed by panel discussion
P.D. Guiney, Retired- S C Johnson & Son, Inc / Molecular and Environmental Toxicology Center
SETAC has maintained and upheld a Code of Ethics for many years. In 2015, the SWC revisited this Code of Ethics as part of their Long Range Planning process and decided to focus on this important topic as a key initiative. A Scientific Integrity Subcommittee was formed under the auspices of the Global Communication Committee. The Steering Committee for this new group met and discussed at least three issues we thought SETAC could significantly influence in

a positive way. First, we felt that there was an increasing lack of personal integrity. Certainly there are institutional biases, mostly in the organizations that are supposedly "protectors of the environment." But underlying that is the question of personal integrity. What are the data really telling us? We need to know; good, bad, and ugly. Secondly, we felt that there was a general lack of understanding industry. Most people have "things" but don't realize all that is involved in the manufacture, distribution, sale, and end-of-life management of "things." There has been an increasing fervor from the business sector of SETAC that there is industry bias. This is an issue that SETAC with its long history of tripartite representation is in a unique position to address. Finally, is the issue of publication bias including what might be called the rise of the on-line journal. Many SETAC scientists get numerous emails requesting that they publish their work in speedy on-line journals at low costs. The on-line publishing world discovered a great "pay to play" model, where almost anyone can get almost anything published in a "peer reviewed" journal. Of course, their Impact Factors are at or below zero, but many times that's irrelevant. Judges don't know about Impact Factors, so a publication in any journal holds a lot of weight in a court of law. When you are in a "publish or perish" institution, any journal is better than none. In addition, because of the plethora of journals, it is becoming nearly impossible to find good reviewers for papers. The current Code of Ethics also mixes two distinct issues: namely personal ethics and scientific ethics. In this context, personal ethics means how someone responds to someone else and scientific ethics means the quality of our research. SETAC has a duty to address both types of ethics. Our medium to longer term, is to separate the two types of (un)ethical behaviour. Thus we are proposing changes and enhancements to both the SETAC Code of Conduct and the Code of Ethics.

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Panel discussion Ctd.

Tendency towards higher complexity in environmental risk assessment of Plant Protection Products: to accept or to avoid?

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Introduction
T. Frische, Federal Environment Agency (UBA) / Section Plant Protection Products

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Issues raised during the brainstorming-workshop "Reflecting on the increasing complexity in environmental risk assessment of Plant Protection Products" organized by the UBA, ANSES, KEMI (12th – 13th November 2015 in Berlin, Germany)
V. Poulsen, ANSES / French Agency for Food Environmental and Occupational Health and Safety

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How to handle decreasing time lines and complex risk assessments of plant protection products - a proposal from KEMI
H. Sundberg, Swedish Chemicals Agency

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Reasons and possible solutions for complexity in risk assessment
F. Streissl, EFSA / Pesticides Unit

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Risk management decisions regarding plant protection products: information needs and regulatory context
K.M. Nienstedt, European Commission - DG SANCO / PPR

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Models are neutral, funding is biased, complexity is real
R. Ashauer, University of York / Environment

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Simplicity, Sense, and Scrutiny in Environmental Risk Assessments of PPPs
P. Kabouw, BASF SE

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Key messages from an NGO perspective
M. Wang, Greenpeace Research Laboratories

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General discussion
T. Frische, Federal Environment Agency (UBA) / Section Plant Protection Products

Persistent and mobile contaminants in the aquatic environment: how to identify, analyse and regulate a potential threat for drinking water resources (II)

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How do chemical and environmental properties affect the retention of organic contaminants in urban watersheds?
A. Parajulee, Y.D. Lei, A. Kananathalingham, University of Toronto Scarborough / Department of Physical and Environmental Sciences; L.W. Yeung, University of Toronto / Chemistry; A.O. De Silva, Environment and Climate Change Canada; C.P. Mitchell, University of Toronto Scarborough / Department of Physical and Environmental Sciences; F. Wania, University of Toronto Scarborough / Physical and Environmental Sciences
Prominent characteristics of the urban landscape include impervious surface coverage and sewer networks that both serve to increase runoff delivery to streams and decrease infiltration. This can lead to rapid, often untreated inputs of contaminants to water bodies during snowmelt and rainfall. Of particular interest are how chemical properties and environmental properties interact during such events to influence contaminant delivery to streams. This study investigates the relative importance of chemical properties, urban infrastructure, and physiography on the transport of a broad range of organic contaminants in urban Canadian watersheds. In Toronto, Canada, we have three sampling sites in each of two watersheds that differ widely in their degree of urbanization, and hence the types and configurations of their land cover and city infrastructure. We measured dissolved and suspended sediment-bound streamwater concentrations of polycyclic aromatic hydrocarbons (PAHs), neutral and acidic pesticides, and benzotriazoles during two rainfall events and one snowmelt event. We also concurrently measured suspended sediment, dissolved and particulate organic carbon, and electrical conductivity. Preliminary results from one rainfall event show peak concentrations of particle-bound PAHs that are 20-94 times higher, and area-normalized fluxes 6 to 24 times higher, at the sites in the more urbanized watershed relative to the largely agricultural watershed. Furthermore, while these peak concentrations increase downstream in the urbanized watershed, there is little to no change in peak concentrations in the other watershed. The total watershed particle-associated PAH loading to Lake Ontario was also 25 times higher. We will additionally present the results of ongoing sample analyses that answer the following questions arising from these initial findings: (1) What drives the higher concentrations and loadings in the highly urbanized watershed? The explanation could be one or both of the following: first, that the urbanized watershed has greater within-watershed sources of PAHs and second, that the mostly agricultural watershed has a greater retention capacity for PAHs. (2) Are the observed patterns consistent across snowmelt and rainfall events, for all analytes, and why or why not?

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Screening of organic contaminants in raw and drinking water from source to tap
L. Ahrens, Swedish University of Agricultural Sciences (SLU) / Dept of Aquatic Sciences and Assessment; R. Troger, Swedish University of Agricultural Sciences / Department of Aquatic Sciences and Assessment; P. Klöckner, Swedish University of Agricultural Sciences SLU; J. Lundqvist, Swedish University of Agricultural Sciences / Department of Biomedical Sciences and Veterinary Public Health; A. Rosenmai, T. Le Godec, Swedish University of Agricultural Sciences; A. Oskarsson, SLU Dept Biomed Sci Vet Publ Health / Dept of Biomed Sci and Vet Publ Health; K. Wiberg, Swedish University of Agricultural Sciences / Department of Aquatic Sciences and Assessment
The occurrence of synthetic organic compounds (OCs) in drinking water can pose a threat to human health. In this study, water samples were collected from drinking water source areas, inside a drinking water treatment plant (DWTP) and in finished drinking water from the surroundings of Uppsala, Sweden. The water samples were examined for 178 residues of pharmaceuticals and personal care products (PPCPs), perfluoroalkyl substances (PFASs) and pesticides. The contaminants were extracted using large volume solid phase extraction (SPE) and analysed using liquid chromatography coupled to a time-of-flight spectrometer (LC/TOF-MS). The main source of the targeted OCs was found to be a sewage treatment plant (STP) effluent. Concentrations decreased in two lake samples (lake Ekoln and Görväln) connected to the DWTP due to dilution effects and potential degradation. Within the DWTP, concentrations remained stable and contaminants were not removed efficiently with the conventional treatment

techniques of flocculation, sand filtration, granular activated carbon (GAC) and disinfection with UV light and chloramine. A pilot plant with membrane technology is ineffective as well, except when coupled to new GAC filter. Overall, the occurrence of contaminants in drinking water emphasis that more research is needed to develop appropriate and cost-effective treatment method for the removal of OCs and to improve our knowledge about their risks for human health and ecosystem.

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Advanced water treatment assessment and novel monitoring techniques
S. Kools, KWR Watercycle Research Institute; K.A. Baken, KWR Watercycle Research Institute / CWG; I. Brüning, IAWR; G. Stroomberg, RIWA River Water Works
To control the emission of chemicals in the environment and to meet the requirements of the WFD, emissions of chemical compounds are more and more regulated, for example by the REACH and other substance specific regulations. Next to that, emission point sources of WWTPs are being upgraded towards the elimination of chemicals of emerging concern (CECs). More specifically, in the Rhine area, several communal waste water treatment plants aim to increase the removal of these CECs. Here, additional treatment steps such as advanced oxidation processes (AOPs) and/or in combination with Granular Activated Carbon (GAC) are being considered or have been implemented. Numerous studies describe the successful removal of substances in waste water, hence higher removal rates by these treatment steps. While the intention is to lower emissions of CECs, resulting transformation products may be overlooked. Concise information on the formation and toxicological relevance of transformation products is lacking and it is unclear to which extent transformation products contribute to overall toxicities of treated waters. This review will collate existing information on the risks of AOPs in the light of large scale applications of these new waste water treatment techniques. Also, the review will identify research questions that arise, specifically the impact of the formation of polar persistent transformation products on drinking water production. The review will highlight existing and novel monitoring techniques, both to identify toxicity as to recognize unknown and known chemicals. As drinking water utilities apply similar techniques for the production of safe and healthy tap water, information may be usable for different water managers and researchers.

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Application of the novel Orbitrap-based gas chromatography-mass spectrometry technology to characterize emerging disinfection by-products in disinfected water.
C. Postigo, IDAEA, CID-CSIC / Environmental Chemistry; C.I. Cojocariu, Thermo Fisher Scientific; S.D. Richardson, University of South Carolina; P. Silcock, Thermo Fisher Scientific; D. Barcelo, IIQAB-CSIC / Dept Environmental Chemistry
About 50% of the halogenated material formed during water disinfection is still unknown[1]. Several chemical classes of low molecular weight **iodinated disinfection by-products (iodo-DBPs)** have been reported to be within the aforementioned unidentified fraction. The presence of iodo-DBPs in drinking water is of concern, because these compounds have been found to be in some cases *more genotoxic and cytotoxic* than their corresponding brominated and chlorinated analogues[1,2]. Recent developments in gas chromatography (GC)–mass spectrometry (MS) allow the use of high resolution-accurate mass (HRAM) Orbitrap mass analyzer to further explore and characterize the volatile and semi-volatile compounds present in the disinfection by-product (DBP) mixtures, providing the resolving power, mass accuracy, and sensitivity required for these tasks. In this context, the main goal of the present work was *to apply the GC Orbitrap MS technology to characterize iodo-DBPs in chlorinated and chloraminated DBP mixtures*. To this end, chlorinated and chloraminated DBP mixtures were generated from a reference aquatic natural organic matter (NOM) solution, i.e., Nordic Reservoir NOM, fortified with bromide (500 ppb) and iodide (50 ppb), and Llobregat river water, and analyzed with a GC-Orbitrap MS. Iodo-DBP characterization was based on HRAM data and fragment rationalization. The workflow applied allowed identification of up to 11 different iodo-DBPs in the DBP mixtures analyzed, including one new iodo-DBP. Overall, it could be also observed that iodo-DBPs were formed to a larger extent after *chloramination* of waters; however, the type of NOM, in terms of aromaticity, and the bromide and iodide content the water were found to be relevant factors affecting the formation of these compounds during disinfection treatments.
Acknowledgments: CP acknowledges support from the EU 7th Seventh Framework Programme (FP7/2007-2013) under grant agreement n° 274379 (Marie Curie IOF). This work has been financially supported by the Generalitat de Catalunya (Consolidated Research Groups "2014 SGR 418 - Water and Soil Quality Unit" and 392 2014 SGR 291 - ICRA) and by the EU's FP7 under grant agreement no. 603437 (SOLUTIONS). The EU is not liable for any use that may be made of the information contained therein.
[1] S.D. Richardson, M.J. Plewa, E.D. Wagner, et al. (2007) *Mutat. Res.* 636, 178-242 [2] S.D. Richardson, F. Fasano, J.J. Ellington, et al. (2008) *Environ. Sci. Technol.* 42: 8330-8338

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Do cytostatic drugs reach drinking water? The case of mycophenolic acid
H. Franquet, Environmental Chemistry; F. Ventura, IDAEA-CSIC; M. Boleda, Aigues de Barcelona / Organic Chemistry Department; S. Lacorte, IDAEA-CSIC / Environmental Chemistry
Mycophenolic acid (MPA) has been identified as a new river contaminant according to its wide use and high predicted environmental concentration in the river (PEC_{river}). In Catalonia (NE Spain), its consumption ranged from 4.7 to 4.9 tons during 2010-2012, which represents 40% of the total drugs administered, and the calculated PEC_{river} was 77.4 ng L^{-1} [1]. Therefore, the aim of this study was to monitor the impact of MPA in a drinking water treatment plant (DWTP) that collects water downstream Llobregat River (NE Spain) in a highly densified urban area. An UPLC-MS/MS method was developed and validated to quantify this compound in water samples. During a one week survey MPA was recurrently detected in the DWTP intake (17 - 56.2 ng L^{-1}). The presence of this compound in river water was associated to its widespread consumption, high excretion rates and low degradability. As MPA was always detected after 7 consecutive days, its fate in waters at each treatment step of the DWTP was studied and complete removal was observed after pretreatment with chlorine dioxide. In addition, degradation experiments were performed using ClO_2 at different concentrations and degradation products were identified. MPA does not reach drinking water considering the current treatments carried out in this particular DWTP. However, its reiterative presence associated with its consumption in anticancer treatments is of relevance to highlight the importance of monitoring this compound.
[1] Franquet-Griell, H., Gomez-Canela, C., Ventura, F., Lacorte, S., 2015. Predicting concentrations of cytostatic drugs in sewage effluents and surface waters of Catalonia (NE Spain). Environ. Res. 138, 161-172.

Pushing nanoparticle studies to the limit - working at environmentally relevant concentrations and with complex matrices (I)

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Detection and quantification of Carbon Nanotubes along the life cycle, using a thermo-optical method
A. John, Institute of Energy and Environmental Technology IUTA eV / Air Quality   Sustainable Nanotechnology; M. Renker, Institute of Energy and Envionmental Technology eV   IUTA; B. Stahlmecke, Institute of Energy and Environmental Technology IUTA eV / Air Quality   Sustainable Nanotechnology; T.A. Kuhlbusch, Institute of Energy and Environmental Technology e.V. - IUTA / Air Quality   Sustainable Nanotechnology; C. Nickel, Institute of Energy and Envionmental Technology e.V. - IUTA / Air Quality   Sustainable Nanotechnology
Carbon nanotubes (CNT) are already used as additives to various structural materials, due to their extraordinary thermal conductivity and mechanical and electrical properties. A release during production, handling, usage, transport or end of life seems possible. Therefore, the detection of CNT along the whole life cycle in different compartments is very important to asses a potential risk for human health and the environment. Depending on the application and release mechanisms CNT may enter different environmental compartments in varying concentrations. Thus, different requirements for the sample preparation and detection have to be considered. One main challenge dealing with the detection of CNT in the different environmental compartments is the high background concentration of natural carbon based materials and the differentiation between CNT and these materials. By using a thermo-optical method a differentiation between different types of CNT and natural carbon based materials and CNT was possible. For this, we adapted a thermo-optical carbon analysis which is routinely used to determine elemental carbon from filters probed with e.g. ambient or workplace air. This method determines total carbon (TC) and uses a specific temperature protocol to fractionate TC into organic carbon (OC) during first temperature steps in pure helium and elemental carbon (EC) during the second temperature steps in a helium/oxygen atmosphere. For the detection of CNT an established NIOSH5040-like protocol „quartz.par" was modified. Two additional temperature steps were integrated in the low temperature EC section to achieve a good differentiation between CNT and natural carbon based materials, like humic acids. During this presentation results for different life cycle stages are presented, starting with the release of CNT in air from products during mechanical stress, the detection of CNT in water and soils, the uptake into organisms and the release of CNT during incineration at the end of life. The results were generated within the CarboLifeCycle project which was founded from the German BMBF, grant 03X0114D.

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The devil is in the details: Tomography to characterize nanoparticle-plant interactions at low ppm exposure concentrations
F. Schwab, CEREGE / Interfast   Transferts; A. Avellan, CEREGE; A. Turner, Duke University; C. Santaella, CNRS/CEA/Aix-Marseille Université / Bioscience and biotechnology Institute of Aix Marseille; J. Bottero, Labex Serenade/CEREGE/Aix-Marseille Université; J. Rose, CEREGE / Interfaces Transferts; C. Levard, CEREGE; M. Auffan, CEREGE / International Consortium

for the Environmental Implications of Nanotechnology
The current understanding of the uptake processes of engineered (nano)materials (ENMs) in plants strongly relies on two-dimensional visualizations of (nano)particles in or on plant tissue, and quantitative analyses of plant digestates. Most of these techniques require the plants to be exposed to very high ENM concentrations (tens to hundreds of mg kg^{-1}), due to the highly complex background matrix of plant tissue, and the low ENM uptake rates of many plants. Such 2D visualizations and uptake concentrations provide valuable information on the mechanisms of acute ENM toxicity, and on ENM affinity to plant tissue. However, it is challenging to use 2D-imaging and digestate analyses to answer questions on the extent and mechanism of internalization of ENMs, or on ENM-plant interactions at environmentally more relevant, subacute exposure concentrations in complex matrices. Therefore, we developed, tested, and validated minimally invasive 3D X-ray micro- and nano-tomography methodologies to semi-quantitatively analyze the nanoparticle distribution and anatomical adaptations to the exposure of ENM in higher plants. Using *Medicago sativa* and *Arabidopsis thaliana*, we studied the ENM distribution of non-dissolving (-/+ nano-Au) nanoparticles. To broaden the understanding of the physiological adaptations of the plants to the ENM exposure, 2D µ-X-ray fluorescence spectroscopy (µXRF) was used. The results show that plants exposed to non-dissolving nano-Au, even at subacute concentrations in the low mg kg^{-1} range, exhibited considerable anatomical changes and adaptations in *M. sativa*. In *A. thaliana*, positively charged nano-Au accumulated in a greater extent preferentially in the border cells of the root cap featuring negative functional groups, and negatively charged nano-Au accumulated to a lesser extent in the apoplast of exposed roots. These findings demonstrate the suitability of 3D X-ray micro- and nanotomography for the analysis of ENM distribution in plant tissue at low ppm exposure concentrations, confirm previous hypotheses about the protective effect of root border cells against positively charged ENMs, and reveal previously unknown changes of the root anatomy of agriculturally relevant legumes upon ENM exposure. [1] Avellan *et al.* in preparation. [2] Schwab F, Zhai G, Kern M, Turner A, Schnoor JL, Wiesner, MR. 2015. Barriers, Pathways and Processes for Uptake, Translocation, and Accumulation of Nanomaterials in Plants—Critical Review. Nanotoxicology early online 1-22.

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Overview of advanced data treatment techniques in spICP-MS using Nanocount
G. Cornelis, University of Gothenburg / Department of Chemistry and Molecular Biology
Single particle ICP-MS has grown fast in recent years as a technique to analyse nanoparticles size and number concentration in particularly difficult samples and at environmentally relevant concentrations. Much of the effort has been in developing techniques for robust data treatment, which is required at different stages to arrive from raw, time-dependent data to a particle size distribution (PSD). The current work presents an overview of previously developed data treatment techniques for particle/dissolved signal discrimination and nebulisation efficiency. The techniques are compared with the FAST spICP-MS approach discussing how such data can be processed and the limits of several approaches. Moreover, a new drift correction procedure is presented, in which longer term drift is corrected. Advanced data treatment techniques offer a number of opportunities not available when using commercially available software preovided by ICP-MS manufacturers. Most notably, reductions in false negatives and positives are to be expected, which is especially relevant when measuring relatively low particle numbers. However, the advanced techniques require much user intervention in the form of expert knowledge, a situation that is probably preventing their routine application.

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Optical nanoparticle analysis for size and shape at environmentally relevant concentrations
S.R. Thompson; J. Yuan, University of York / Phyics; A. Boxall, University of York / Environment Department
In recent years, environmental nanoparticle research has shifted away from understanding the specific effects of specific nanoparticles on specific organisms at unrealistically high concentrations, and towards more mechanistic studies aimed at discovering the causes of nanoparticle toxicity rather than the effects, at more environmentally relevant concentrations. One of the major challenges with running these studies is the difficulty of confirming the state of nanoparticles once they've been released into the system – at environmentally relevant concentrations the only commercially available system capable of making such measurements is a single-particle ICP-MS setup. This is large and non-portable, expensive, and isn't capable of inferring much about the shapes of the particles it measures. We present a novel optical scattering device, which can be built for under £5,000 (€7,000, US$7,500), miniaturised to fit inside a medium-sized suitcase, operated in-situ using a laptop and an automotive battery, and which can take statistically robust measurements of nanoparticles at concentrations 2 orders of magnitude lower than current optical techniques, allowing it to rival detection thresholds in current sp-ICP-MS setups. The system is also capable of taking simultaneous size and shape measurements of nanoparticles.

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Single-particle ICP-TOF-MS provides elemental ratios on an individual particle level for the identification of cerium oxide nanoparticles at environmentally relevant concentrations
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Analytical methods to detect, quantify and characterise CeO_2 NPs in soils and surface waters are urgently needed to assess environmental exposure and risk of these materials. The main challenge in the detection of engineered CeO_2 NPs in environmental matrices is the presence of natural Ce-containing minerals of similar size range at concentrations several orders of magnitude above expected levels for engineered CeO_2 NPs. A promising approach to identify engineered CeO_2 NPs against the natural background is to take advantage of the different element ratios of natural versus engineered Ce-containing NPs. For example, the Ce to La ratio is expected to be relatively stable at about 2:1 in natural environments, while engineered CeO_2 NPs show a Ce:La ratio of about 4000:1. However, due to the much higher natural Ce-levels compared to engineered CeO_2 NP concentrations in the environment, the sensitivity gained from the element ratio alone is too low to yield any detectable results when performing conventional bulk analysis on soil samples. We therefore have to move towards an assessment of the elemental ratios on an individual particle level. We here present a new approach, using a prototype ICP-TOF-MS instrument, coupling a conventional ICP-MS with a time-of-flight (TOF) instrument, enabling the simultaneous measurement of multiple elements at high time resolution. The applicability of this method for the detection of engineered CeO_2 NPs in complex samples is demonstrated on a series of natural soils spiked with different concentrations of CeO_2 NPs. Our results show, that we can clearly differentiate between Ce- and La-containing natural particles and Ce-only containing engineered nanoparticles using the ICP-TOF-MS in single-particle mode. Furthermore, a more advanced data analysis method based on a Machine Learning approach makes it possible to improve our detection by establishing elemental "fingerprints" of natural and engineered Ce-containing NPs based on 30 selected elements. This provides a more reliable analysis, especially for particle signals close to the detection limit. In this way we are able to confidently detect engineered CeO_2 NPs at ppb concentration levels. The new method represents an important advancement for the detection of ENPs in complex matrices at environmentally relevant concentrations and can likely be applied to a wide range of other ENPs in the future.

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Application of single particle ICP-MS for the detection of nanomaterials in complex environmental samples
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Regulatory efforts rely on nanometrology for the development and implementation of laws regarding the incorporation of engineered nanomaterials (ENMs) into industrial and consumer products. Therefore, advanced analytical techniques combined with appropriate sample pre-treatment methods are necessary for the isolation and analysis of nanomaterials in environmental samples. Environmental nanometrology unavoidably deals with materials of poorly-defined properties and in complex matrixes, in which nanomaterials may undergo significant transformations. It is therefore imperative to develop appropriate analysis protocols to understand the environmental dynamics of these materials. Single particle inductively coupled plasma mass spectroscopy (spICP-MS) has been suggested as a powerful tool for routine nanometrology efforts. However, this technique is not yet developed to its full potential and method development is required for different ENMs and natural systems. In this work, we apply several spICP-MS methods for the detection of titanium dioxide ENMs in natural surface waters and copper oxide ENMs in colloidal extracts from natural soil samples. In the former case we aim at overcoming the challenge posed by high Calcium concentrations and the isobaric interference of ^{48}Ca isotope on the most abundant ^{48}Ti isotope; although the natural abundance of the ^{48}Ca isotope is relatively low (0.19 %) compared to ^{48}Ti (73.8 %), Ca concentrations in surface waters are typically more than three orders of magnitude higher than Ti. In the latter case we adjust a spICP-MS method on the detection of CuO ENMs in soil colloid extracts, based on the characteristics of the soils. Overall, our work demonstrated the great potential of spICP-MS for the analysis of ENMs in

real-world environmental samples, while highlighting the limitations and challenges for two case studies. TiO_2 ENMs were successfully measured in the presence of high Calcium concentrations; however, this method is not able to measure TiO_2 particles smaller than 80 nm. CuO ENMs were also detected in spiked colloidal extracts; we choose a rather conservative approach for particle detection with the goal of avoiding falsely identifying background signal as engineered CuO NP particles. In this context, the need for comparing and correcting detected particles spikes against the presence of false positive spikes is also discussed.

Ecological traps for wildlife driven by pollutants

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Eutrophic wetlands receiving effluents from wastewater treatment plants may act as ecological traps for endangered waterfowl

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Animals can select habitat according to characteristics of the environment that correlate with better survival and reproductive success. In rapidly changing environments, these natural preferences can become ecological traps if the selected aspects of the habitat are accompanied with adverse environmental factors that affect individual fitness. Pollutants can be one of the important drivers of ecological traps nowadays. One of the potential scenarios that matches the concept of ecological trap are the eutrophic wetlands receiving effluents from wastewater treatment plants. These wetlands are highly productive and therefore attractive for waterbirds, especially those feeding on aquatic invertebrates like the white-headed duck (*Oxyura leucocephala*). These wetlands may also receive a myriad of pollutants from urban and industrial sewage that can accumulate and affect the ecology of the wetland and/or the health status of the waterbirds living there. Here we discuss the potential effect of these ecological traps for the white-headed duck and the marbled teal (*Marmaronetta angustirostris*), which are globally endangered species under threat in their remaining breeding areas such as Spain. The studied birds were marbled teals (n=28) and white-headed ducks (n=12), but also the invasive ruddy duck (*Oxyura jamaicensis*) (n=13) and their hydrids (*Oxyura leucocephala x jamaicensis*) (n=5) Organochlorine pesticides and PCBs were measured in liver and adipose tissue samples after extraction with hexane, clean-up with sulfuric acid and analysis by GC-ECD. Birds of the genus *Oxyura* showed higher organochlorine levels than marbled teal, especially in adipose tissue. No differences in organochlorine levels were found between each *Oxyura* species and their hybrids, between geographical areas (Andalusia, Valencia or Central Spain), by sex or by age. Several potential biotic (i.e. pathogens) and abiotic agents (i.e. chemical pollutants) may drive negative influences on the populations of endangered waterfowl through a mechanism in eutrophic wetlands that can be defined as an ecological trap. In addition to the mortality caused by botulism outbreaks and lead poisoning, white-headed duck shows a low reproductive outcome that could be caused by pollutants accumulated in the eutrophic lakes frequently selected by this species.

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Can POPs contaminated sites be ecological traps for top predators?

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Numerous contaminated sites are documented in North America and many other parts of the globe. Among the main contaminants at such sites are legacy persistent organic pollutants (POPs) such as PCBs and organochlorine insecticides. Wildlife, including top-predatory birds and mammals may be attracted to such sites as the habitat often appears suitable. In the process, species such as river otters, can bioaccumulate POPs with potential consequences on health and fitness. Previously we used fecal samples collected from river otter communal marking sites (latrines)at the federal contaminated site of Victoria Harbour, BC, Canada, reported levels of PCBs that exceeded 9 mg/kg lipid, a published criteria for reproductive impairment in mustelids. Following up on that study, we again used scats to show variation in PCB exposure in time and space, and the population implications. In the present study again we used fecal sampling but combined with radio-telemetry to further investigate the exposure and to infer population level effects in river otters in that contaminated system. We also examine the question of whether the harbour constitutes an ecological trap situation. The implications of population structuring relate to the extent and impacts of contaminant exposure based on where the otters spend their time foraging and whether or not they are able to sustain a population in a contaminated site environment.

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Are cities ecological traps for birds because of pollutants?

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Cities have higher temperatures, louder noise, lower parasite prevalence and higher levels of pollutants than rural habitats. They are considered as ecological traps because studies dealing with the impact of urbanization on bird fitness have shown that breeding parameters are affected. Among the mechanisms that may explain the responses of birds to urbanization, the impact of pollution has received little attention although cities are characterized by the circulation of many different pollutants. We hypothesized that NO_2 concentrations in the air and/or concentrations of non-essential trace elements (TEs) in blood were positively correlated to reduced reproductive outputs and/or nestling body condition in urban populations of Great tits (*Parus major*) compared to forest ones, while concentrations of essential elements in blood were negatively related to breeding outputs and/or individual performances if their concentrations exceeded threshold values or, on the contrary, if they did not meet physiological (homeostatic) requirements. Two cities and two forests were studied in eastern France. Each site received 150 artificial nestboxes that were inspected from March 20th to record breeding success from 2012 to 2014. In 2013, passive NO_2 samplers were put on nestboxes and the concentrations of 25 essential and non-essential elements were determined in 13-day old nestlings. In urban sites, breeding success, nestling growth rate and body condition were reduced. Atmospheric concentrations of NO_2 significantly differed between the four sites but no significant correlation was detected between NO_2 and biological parameters. Based on the results of a PCA on metal concentrations, we used the coordinates on the first axis as an index of richness in essential elements (REE), with negative values indicating high concentrations in essential TEs, and positive value indicating low concentrations. Brood body mass was positively correlated with REE. No other significant correlation was detected between REE and biological parameters. Our study brings new data about plasmatic concentrations of various essential elements in birds from urban and forest habitats and their positive relation to nestling body mass and condition. To our opinion, this is a clue to suggest that food availability and/or quality may be a causal mechanism for a reduced growth of nestling birds in urban areas, an issue that would need further investigations in urban ecology and in ecotoxicology in general.

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Short-term and residual effects of pesticides on mosquito oviposition site selection and larval performance, and on aquatic community structure

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Pesticides can have immediate toxic effects on targeted pest species as well as on non-target species for broad-spectrum pesticides. Broad-spectrum pesticides may also have positive effects on mosquitoes after the pesticide no longer has direct toxicological effects by altering the community structure – i.e. by reducing abundances of mosquito competitors and predators, and via a trophic cascade, increasing food resources for mosquito larvae. Moreover, in some cases, pesticides can act as an ecological trap for some taxa, attracting colonizer insects to habitats that are ultimately lethal for them. The present study was undertaken to assess mosquito oviposition habitat selection, mosquito larval performance and community structure alterations after pesticide applications. The experiment was conducted in outdoor mesocosms assigned to one of four treatments: (1) control – no pesticides; (2) *Bacillus thuringiensis israelensis*, a mosquito larvicide well-known for its selectivity for Nematoceradipterans; (3) temephos, a mosquito larvicide of the organophosphate class; (4) pyriproxifen, a pyridine-based pesticide of the Insect Growth Regulator (IGR) class. All three of these pesticides have short activity periods. Invertebrates were sampled 2 days before treatments, 4 and 7 days after treatment and then, every two weeks for a period of 2 months. Environmental parameters (water temperature, pH, conductivity, chlorophyll *a*) were measured in every mesocosm on each sampling date. Mosquito oviposition was monitored every 3 days. After applications, the number of egg rafts decreased in all the treated pools, but remained stable in the controls. Species richness and invertebrate abundance were also initially strongly reduced in the broad-spectrum pesticides (temephos and pyriproxifen) when compared to both the control and to the selective *B. thuringiensis israelensis* treatment, leading to an increase of chlorophyll *a* concentrations. One month after the treatment, mosquito oviposition was higher in the pyriproxifen-treated pools, in comparison to other treatments. However, mosquito larval survival remained lower in pyriproxifen-treated pools, even though pyriproxifen was not detected in the water samples 7 days after the application. Our results suggest that pyriproxifen appears to provide an ecological-trap, attracting mosquito oviposition due to altered community structure (fewer antagonists and more algal resources) but causing high mosquito larval mortality.

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Are treated seeds an ecological trap for birds?

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Due to reductions in winter food resources, newly sown cereal seeds have become a key component of many bird species' diet, but these seeds are often treated with pesticides that may cause toxic effects. In order to complete an appropriate risk assessment, data on treated seed toxicity need to be combined with information about the risk of exposure of birds in the field. The aims of the present work are to characterize the exposure of farmland birds to pesticide-coated seeds in the wild, and to estimate the risk of poisoning of animals as a consequence of such exposure. To do this we analysed crop and gizzard contents of hunted partridges (n=189) to detect residues of pesticides used for seed treatment. Moreover, we measured the contribution of cereal seeds in the winter diet of partridges in order to assess the potential risk of exposure to pesticide-treated seeds. We also studied the abundance of pesticide-treated seeds available for birds in the field (n=48), the pesticides and their concentrations in these treated seeds, and the bird species that feed on them. Seed availability was influenced by the sowing method and the location within the field. Regardless of the sowing technique, there were more seeds on the surface in the headland (43.4 ± 5.5 seeds/m^2) than in the field centre (11.3 ± 1.2 seeds/m^2) (t_{151}=-4.67, p< 0.001). Six pesticides, mostly fungicides, were detected in the seeds collected from recently sown fields. 55.2 % of the seed samples contained detectable levels of pesticides, with tebuconazole being the most frequently found active ingredient. During the sowing period, 31 species were observed feeding on sown cereal seeds. We detected pesticide residues in the digestive content of 32.3% of the analysed red-legged partridges, including insecticides in a 3.7% of the analysed samples and fungicides in a 29.6% of them. As observed in seeds, tebuconazole was the most frequently detected pesticide (19.1% of the samples). Winter cereal seeds represented a 53.4% of the biomass found in the digestive content of red-legged partridges. The present study demonstrates that the use of pesticide-treated seeds constitutes an important way of exposure of farmland birds to pesticides, and that there exists a potential risk for these birds to suffer toxic effects from ingestion of treated seeds in the wild.

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Mange, anticoagulants, and felids: Investigating immune response to low dose brodifacoum exposure

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Anticoagulant rodenticides are used worldwide to control pest rodents in urban, suburban, and agricultural settings. This widespread use poses potential risk of exposure to non-target species. A study of bobcats and mountain lions near Los Angeles, CA detected a correlation between anticoagulant exposure and mange. Exposure to brodifacoum has thus been proposed as a potential mechanism for this effect in felids, though domestic cats have been shown to have high tolerance for anticoagulants. More research is needed to determine potential effects of brodifacoum that a cat would be exposed to by consuming non-target rodents. In this study, cats were offered 0.05 mg/kg brodifacoum once per week mixed into canned food and vaccinated with irrelevant antigen (ovalbumin, OVA) and adjuvant starting at week 1, with booster injections on weeks 4 and 8 to test effects of brodifacoum on recall antigen immune responses. Blood was collected weekly beginning on day 0 to additionally assess PBMC proliferation following exposure to ConA, KLH, and OVA. Whole blood was frozen for brodifacoum residue determination. None of the cats exhibited any clinical abnormalities during the study. Cats in both groups gained an average of 100g with no significant weight differences between the groups at the conclusion of the study (control= 4.06± 0.68 kg, brodifacoum= 4.08± 1.16 kg). Cats did not show any overt signs of anticoagulant intoxication for the duration of the study. Brodifacoum residue levels were determined from liver biopsies taken on day 77. Brodifacoum residues were 1806 ng/g in treated cats (stdev 105.7 ng/g) and not detected in control subjects (LOD 5.8, LOQ 19.2 ng/g). Brodifacoum concentrations in blood were determined weekly over the course of the study. Clotting times, as measured by PT, between the control and brodifacoum groups were not significantly different at any of the timepoints through week 6 post toxicant exposure. Delayed type hypersensitivity tests were not significantly different between control and brodifacoum dosed groups in the first three time points tested. Completion of this investigation will assess gross impacts of environmentally realistic brodifacoum exposure on humoral and cell mediated immunity against foreign antigen exposures in domestic cats. Additional studies will need to be conducted to determine if non-target exposure to brodifacoum could increase susceptibility to potential pathogens. \n

An in silico modelling perspective to advance hazard assessment of aquatic ecotoxicology

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Evaluation of currently available methods for determining log Kow values of surfactants

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The n-octanol/water partition coefficient (log Kow) is a key parameter in chemical environmental studies as it determines the environmental fate and bioavailability and thus toxicity of a compound. Due to their unique properties, measurement of log Kow values of surfactants is a technical challenge. The traditional 'shake-flask' method (OECD 107) is no longer considered appropriate. Here we compare log Kow values of surfactants were compared using a range of existing experimental and predictive methods which have been used by lead registrants in REACh phases 1 and 2. However, there are concerns that these methods have not been fully validated for surfactants and may not be applicable due to the specific phase behaviour of surfactants, leading to unreliable or unrealistic values. This is complicated by the fact that the solubility is not properly defined for surfactants since they will form a micellar phase above the Critical Micelle Concentration (CMC) up to relatively high concentrations. In the light of this, logKow values were generated for a select set of 12 surfactants. Side-by-side comparisons of values derived using different experimental methods compared against predicted values identified whether certain existing methods give similar results for the same surfactants and recommendations made.

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New Mechanism of Action definitions based on Molecular Initiating Events and a classification algorithm based on calculated structure properties.

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A good understanding of Mechanisms of Action (MechoAs) and appropriate methods to determine them is crucial for the efficient prediction of ecotoxicity using *in silico* techniques. Different MechoAs will need different parameters or different coefficients to calculate toxicity values. To date, several classification methods, based on molecular structure analysis, exist to determine the Mode of Action (MoA) in aquatic species. The most commonly used one in regulatory ecotoxicology is the scheme proposed by Verhaar and co-workers (1992). The MoAs defined according to this scheme are based on chemical group reactivity and differences in logK_{OW} – logEC50 relationships. However, to be able to derive accurate predictions of modes or mechanisms of action only from structure, a classification is required that is based on Molecular Initiating Events leading to an adverse effect. So we defined a new set of MechoAs, partly based on the work of Russom and co-workers (1997). The approach used in Verhaar's classification algorithm has several limitations, and some classification errors were reported (Ellison *et al*., 2015). This classification algorithm is indeed based on a decision tree identifying 2D structural features that are recognised to lead to a certain MoA, limiting its Applicability Domain. In the present work, an approach to define MechoA for narcotics and beyond was applied using molecular modelling to obtain calculated molecular parameters depending on the 3D structure, such as E(LUMO) and qMaxH. A similar approach has already been implemented with success for a limited set of chemical families (Asadollahi-Baboli, 2012; Schultz *et al*., unpublished work). The results of this new algorithm coupled with the use of more pertinent MechoAs were compared with those obtained with Verhaar's scheme, as implemented in ToxTree software, using a comparison dataset taken from the literature (Ellison *et al*., 2015). With this new method applied to the validation set, we obtained 79% correct classifications, 11% misclassifications, 7% of the substances fell in a zone where two possible MechoAs couldn't be differentiated and the remaining 3% where out of the Applicability Domain of the algorithm. The same test applied to the Verhaar scheme with the same validation set obtained only 48% correct classification, 14% misclassifications and as much as 38% out of the Applicability Domain. Thus work on this new method will be continued.

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Fragment Model to Predict the Rate of Hydrolysis for Organic Compounds

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Hydrolysis as one of the key degradation processes in the environment is considered to be important for a number of classes of organic compounds as carboxylic acid esters and amides, anhydrides, epoxides, isocyanates and isothiocyanates, carbamates, phosphate and thiophosphate esters, alkoxysilanes and halosilanes, and halogenated hydrocarbons. However, the number of available experimental data is rather limited. Computer models are a promising alternative to overcome respective data gaps. The overall hydrolysis is a combination of the reaction with neutral water molecules and acid and base catalysed degradation reactions. These processes will take place to a different degree. Since the acid and base catalysed degradation depends on the pH, the role of the individual processes and thus the overall rate depend on the pH of the aquatic environment. A feasible model approach is to express this rather complex system of reactions in terms of simple pseudo-first-order kinetics. The reaction with neutral water is directly addressed by a pseudo-first-order rate that is a constant for a given chemical and temperature and independent of the pH. Acid and base rates are primarily derived as pH-independent second-order rate constant and then transformed into pseudo-first-order rates by application to a fixed pH and thus to given H^+ or OH^-

concentrations. For the fixed pH the overall rate then can be calculated from the combination of the individual rates. In the presented study, three independent fragment models are being developed for the individual pH-independent rate constants, and the overall rate constants are obtained by putting them together for a particular pH then. The model is implemented and will be publically available after its finalisation in the OSIRIS edition of the software system ChemProp (UFZ Dpt. Ecological Chemistry 2015. ChemProp 6.3 http://www.ufz.de/index.php?en=6738). The training set consists of about 2200 compounds of all considerably hydrolysing compound classes. For ca. 670 substances, rates of the neutral reaction are available, and for the acid and base catalysed reaction there are data for 750 and 1280 chemicals. The models comprise ca. 75, 85, and 125 structural fragments for the estimation of the neutral, acidic, and basic rate. The performance with respect to the overall rate is ca. 0.9 (squared correlation coefficient of regression). The reliability of the model and the comparison to existing approaches will be discussed.

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Development of parameters to predict hydrophobicity and sorption of non-ionic and anionic surfactants

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Octanol-water partition coefficients (K_{ow}) are often used in environmental risk assessment as a measure for hydrophobicity of organic contaminants to predict bioaccumulation, toxicity, and sorption to soil. The K_{ow} value is therefore an important parameter in predictive modelling of environmental fate and exposure of organic contaminants. However, application of the K_{ow} value as a parameter in predictive models for surfactants becomes problematic because of the amphiphilic nature of surfactants. More knowledge about the environmental behaviour of ionized and surface-active substances is therefore required to expand the chemical applicability domain of the currently used predictive models. Sorption of surfactants to polymer-coated fibres can be used as a measure of hydrophobicity. In addition, retention on stationary phases used in liquid chromatography (e.g., C_{18}, HILIC or ion-exchange phases) can be used as a probe to simulate sorptive interactions of surfactants with relevant environmental sorption phases (e.g., membrane lipids, organic matter, etcetera). The applicability of polymer phases and selected stationary phases to determine the hydrophobicity of non-ionic and anionic surfactants and to predict their bioaccumulation in aquatic organisms will be explored in this study based on literature and experimental data from different surfactant groups. The possibility of using such methods as a measure of hydrophobicity will have important consequences for the regulatory evaluation of surfactants within REACH and OSPAR. Results from this study show that fibre-water sorption coefficients and capacity factors on a C_{18} phase show great promise for the prediction of hydrophobicity of non-ionic and anionic surfactants. These values that reflect differences in hydrophobicity can be correlated with available data of bioaccumulation, toxicity or sorption. However, a single parameter or a single polymer or hydrophobic phase will not be sufficient in predictive modeling of sorption phenomena of surfactants to environmental phases. Likely, a series of phases are needed that correspond with specific types of interaction.

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Replacing Fish Acute Toxicity Tests by QSAR Predictions: How much is realistic?

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Alternative methods like QSARs (quantitative structure-activity relationships) can replace some (eco)toxicity testing in regulatory contexts (e.g. REACH). Complete substitution of in vivo animal tests by in silico methods is, however, not realistic. Reliable QSAR predictions from the octanol/water partition coefficient (log K_{ow}) are possible only for certain classes of chemicals, the so-called baseline toxicants. Other chemicals that may reveal excess toxicity and/or act by specific modes of action (MOA) require further assessments. For fish acute toxicity, we derived a scheme to group chemicals as either baseline toxicants (= predictable by QSARs) or as potential excess toxicants (= not predictable by baseline QSARs). This scheme was tested with a new data set obtained with diverse fish species. Only for baseline toxicants, it is possible to predict the fish acute toxicity with sufficient accuracy from log K_{ow} and, hence, valid QSARs can replace in vivo testing. In contrast, excess toxicants and chemicals not reliably classified as baseline toxicants, require further in silico, in vitro or in vivo assessments. The stepwise approach identifies baseline toxicants (true negatives) in a precautionary way, not ignoring possible excess toxicants (true positives). At the same time, we tolerate a certain fraction of false positives, i.e. baseline toxicants without specific effects that may be tested instead of predicted. The way to replace many fish acute toxicity tests by QSAR predictions takes 3 steps: First, we use a classification scheme to discriminate between baseline and excess toxicants. Second, we apply high quality QSARs that meet the requirements for regulatory acceptance to replace in vivo fish acute toxicity tests for baseline toxicants with QSAR predictions. Third, the remaining chemicals, i.e. excess toxicants and chemicals not reliably classified as baseline toxicants, have to be assessed by further in silico, in vitro or in vivo methods. Application of the classification scheme to identify potential baseline toxicants to a new heterogeneous data set obtained with diverse fish species results in about 50% baseline toxicants, almost 30% excess toxicants and nearly 20% compounds not classified. Thus, we can conclude that replacing at least 50% of the fish acute toxicity tests by QSAR predictions is realistic.

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Can we venture beyond experimental limits using HA-QSAR methodology?

P. Bicherel, P. Thomas, KREATiS

In aquatic ecotoxicology water solubility is considered a key parameter since it provides an indication of how much of a substance can be available for uptake by organisms. In general ecotoxicity of very hydrophobic substances are difficult to measure. They are likely to not have reached equilibrium within the experiment's duration and it is often technically difficult to achieve accurate concentration measurements. On the contrary no quantitative method is available to assess the miscibility of very water soluble substances. QSAR/QSPR modelling is a semi-empiric approach based on measured data. Their applicability domain tends to be limited by the extreme values in the available dataset. In this presentation we consider whether extrapolations beyond the data boundaries can be justified for both miscible and very hydrophobic substances using High-Accuracy QSAR models. On the hand, for "miscible substances" without any experimental quantification of the solubility, the water solubility values predicted by the HA-QSAR model are completely in agreement with the ecotoxicity values even if they cannot be compared to experimental water solubility. At the other end of the scale predicted QSAR values cannot be extrapolated beyond a recognised solubility/log K_{OW} cut-off value since acute tests are too short to reach equilibrium for very hydrophobic substances. Thus, for certain QSAR models, it is not the availability of the extreme datapoints in the dataset that determine the applicability domain, but other factors such as equilibrium with the test duration that will determine whether a substance endpoint value can be accurately predicted or not.

Mechanistic effect modelling for risk assessment: state of the art, applications, use in a regulatory context and future directions (I)

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Selecting mechanistic effect models for environmental risk assessment; the link with protection goals and test protocols

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Virtually all frameworks for environmental risk assessment assess risks by combining the results from an exposure and an effects assessment. Estimation of exposure concentrations relies heavily on mechanistic fate models, but for the effects side, in contrast, focus lies with toxicity testing and descriptive statistical treatment of data. Mechanistic effect models do exist, however, and are gaining increasing interest in a regulatory context. A wide range of effect models exists at different levels of biological organisation (individual, population, community). These models differ in their degree of complexity, in their underlying assumptions about the biological and toxicological reality, in their 'quality' (as assessed from a good-modelling perspective), and in their data requirements, making it impossible to see the wood for the trees. The selection of the most appropriate models cannot be performed in isolation. Model selection is best viewed in conjunction with two other issues: the protection goals and the test protocols. The protection goals, when explicitly formulated, will make it easy to select (or develop) useful models. And subsequently, the useful models should guide the most efficient design for toxicity testing. At this moment, the linkage between these three components (protection goal, model selection and test protocols) is rather chaotic. Protection goals defined in risk assessment regulations are too vague to be of much guidance, models are often developed by modellers out of scientific interest, and existing test protocols are generally quite useless to support parameterisation of mechanistic models. For these reasons, environmental risk assessments needs a more ambitious agenda to redesign the effects assessment, and that requires an integrated evaluation of the three main issues. Model selection cannot be performed by only considering the 'quality' of the models. Instead of focussing too hard on model 'quality', we need to consider that the task of model selection (or development of novel models) is intricately linked to the regulatory protection goals and to the process of designing test protocols. In this contribution, I will provide several example cases to demonstrate how these linkages might work out. I hope that this contribution can help to streamline the fruitful integration of mechanistic modelling into a regulatory setting.

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Optimizing experimental design for calibration of GUTS models

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The General Unified Threshold model of Survival (GUTS) provides a theoretical framework for analysing stressor effects on survival over time through consistent model equations based on different assumptions about the stressor quantification, the compensatory processes and the nature of the death process. In ecotoxicology, stressors are toxicants characterised by a dose metric, e.g. the concentration in the medium surrounding an organism or inside the organism, or by the damage quantity they cause. The key GUTS feature is that mortality is estimated when the dose metric exceeds a certain threshold. Several GUTS flavours can be derived according to the assumption underlying the death process: (i) the threshold is distributed within a population, and when exceeded, the individual dies (individual tolerance, IT); (ii) there is one common threshold for all individuals, and when exceeded, the probability to die increases (stochastic death, SD); (iii) a unification of both previous assumptions (GUTS proper). While more realistic, GUTS proper requires the estimation of one additional parameter. Because environmental risk assessment of chemicals depends on robust estimates of GUTS parameters, we investigated parameter identification for GUTS proper, in relation to the experimental design of 'short-term' laboratory bioassays. In practice, standard survival datasets generally do not contain enough information to estimate all parameters of GUTS proper with sufficient precision. This is because a large number of individuals is required to provide strong information on probabilistic events. Hence, based on simulated datasets we identify appropriate experimental designs suitable to estimate all parameters of GUTS proper with the best possible precision. We show that datasets with a high number of animals per treatment allow for parameter estimation of GUTS proper with reasonable accuracy and precision. Moreover, increasing the number of animals or the duration of the experiment substantially reduce the uncertainty around the median value of the threshold. Nevertheless, general statements about optimisation for any chemical, any species, any test duration and/or any exposure concentration profile remain difficult. As take-home message, to the extent possible, we recommend not to use fixed experimental set-up for GUTS analyses, but rather tailor dedicated designs according to the chemical, the species and/or the research/regulatory question at hand.

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Validation status of TKTD models: predictions of survival after time-variable exposure to pesticides match observations, but not all details
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TK/TD models are supposed to deliver relevant information for environmental risk assessment of pesticides, especially for the extrapolation from toxicity observed under constant laboratory conditions to survival under time-variable exposure, as expected for realistic application scenarios. TK/TD models from the GUTS framework describe the expected survival for a number of individuals being exposed to chemical concentrations over time. These models are structurally simple and consist of three or four equations for all species, and species- and compound-specific information is captured by the model parameters. Standard acute toxicity test results are often the only data being available for model parameterisation and can be used to parameterize the individual tolerance and the stochastic death survival submodels, using scaled internal concentrations as dose metric. For an application in risk assessment, it is required to check the quality of model predictions, or, with other words, to validate parameterised models for specific combinations of compounds and species. This study presents validation results for TK/TD models for some sets of species and compounds, including the neonicotinoids imidacloprid, thiamethoxam and thiacloprid, the benzimidazole fungicide carbendazim, the pyrethroid insecticide cypermethrin, and the organophosphate insecticide and acetylcholine esterase inhibitors chlorpyrifos, dimethoate and malathion, using models that were parameterised on standard acute data and using observed survival of individuals under time-variable exposure as validation data sets. The objective was to give an overview of the validation status of GUTS models and to analyse the validation results for patterns of prediction qualities across different compound classes and species. In short, from the current results no obvious patterns in prediction qualities appear that relate to specific models, species or compounds. Despite the goodness of fit of the temporal dynamics is of limited quality for some datasets, the match of observed and predicted survival at the end of the experiments is acceptable (shown in presentation). This observation indicates that TK/TD models can be used to reduce uncertainties in risk calculations for time-variable exposure time series, as compared to ratio-based methods. Subtle patterns in survival can possibly only be predicted when the model is being calibrated on survival data obtained from more complex exposure scenarios

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Exposure specific species sensitivity - a toxicokinetic-toxicodynamic approach
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Currently in higher tier environmental risk assessment of chemicals, species sensitivity distributions (SSD) are used to describe and to quantify differences in sensitivity among species. When available for multiple species, no-observed-effect-concentrations (NOECs) or ECx, (representing x% of an affected population) usually derived from acute and chronic toxicity tests under constant exposure, are being used to calculate SSDs. In turn, these SSDs are used to extract 'safe' community effect thresholds in the form of an HC5, i.e. the concentration at which 5% of the species are affected. In current ERA practice, the ratio of effect measures and exposure, such as the maximum predicted exposure concentration (PECmax) or the time weighted average (TWA), are being employed to characterize risk ignoring that, depending on environmental conditions, the exposure concentrations are highly variable in space and time. Mechanistic models, such as the General Unified Treshold model of Survival (GUTS), take the process leading to an effect into account and can explicitly cope with time variable exposure. We present the outcome of a GUTS workshop held in March 2015 where species sensitivity distributions under time variable exposure have been studied. Our results point towards an unrecognized problem with the widely used SSDs and HC5 values: they could depend on the exposure pattern and differ a lot for exposures with the same time weighted average concentration but different temporal profiles. By using GUTS to analyze and compare species sensitivities, we can overcome the dependence of time and exposure patterns, and extrapolate toxicity to other exposure scenarios. Furthermore, we can develop a more thorough understanding of the underlying toxicity mechanisms by quantifying organism internal toxicity thresholds in conjunction with compensating processes.

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How to map ecological risk assessments of chemicals
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The lack of ecological realism is widely recognised as a limitation in current prospective chemical risk assessment, as is the failure of making variability explicit and transparent. The integration of ecological scenarios with chemical effect models to achieve quantitative ERA promises to increase ecological relevance. Probabilistic environmental risk assessment (PERA) has been suggested as a way to account for spatial, temporal, and environmental variability. Probabilistic plots area new way of presenting ecotoxicological data whilst accounting also for ecologically relevant parameters. They provide an indication of the maximum population-relevant impact of an effect of interest (e.g. biomass reduction) and the prevalence of this impact. Essentially they answer two related questions: How strong is the effect? In how many locations will we see the effect? We discuss some of the challenges and opportunities involved in bringing these new concepts into everyday risk assessment for down-the-drain chemicals. One of the key questions revolves around understanding the protection goal for anthropogenic stressors in specific ecological scenarios, and indeed whether certain scenarios require specific modified protection goals. Once the specific protection goal has been established, a metric to suit both the specific ecological scenario and protection goal needs to be defined and agreed. The selection of this endpoint must be carefully considered as different options will lead to different interpretation. We present a framework to integrate probabilistic approaches with mechanistic effect models to assess variable chemical and environmental scenarios. We present a hypothetical case study risk assessment for an ingredient used universally in all laundry products across Europe and illustrate the potential benefits of the framework. To do so, we use an individual based model integrating a dynamic energy budget model to assess the potential impact of chemicals associated with local environmental characteristics. We then map the outcomes based on probabilistic plots and on potential policy makers' decisions of the maximal ecologically acceptable impact and the maximal prevalence of this impact. This new framework has the potential to better present ecologically relevant risk by using integrated biological endpoints and to aid more transparent risk communication.

Application of a coordinated OMICS research program and data into regulatory frameworks: case-studies and perspectives

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SETAC Global Advisory Group OMICS: Cutting-edge science to solve real world problems
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Governments around the world are passing new legislation to regulate chemical substances considering their potential impacts on both human and environmental health. These are critical and complex rules, designed to better ensure public

safety, a healthy environment and functioning ecosystems. However, there are huge scientific and logistical constraints in determining risks for an ever-increasing number and diversity of products introduced to markets. Standard operating procedures for toxicity testing are typically prescriptive and have not fundamentally changed, being too expensive and time consuming to keep pace with requirements.To fully implement these policy directives, we require equally novel, revolutionary and coordinated scientific approaches that are rapid, cost effective, and consistent with global ethical standards for the treatment of animals.A proposed solution is to apply OMICS approaches. Key questions that arise include the reliability and cost of these and the extent to which OMICs measurements can be tied to mechanisms of toxicity and adverse outcome pathways.Following several activities/discussions organized within SETAC in the last decade, the Global Advisory Group OMICs (**OMICs AG**) was recently being established. Here, we will be rolling out the mission, objectives and goals we propose to reach and how we plan to achieve them.**The OMICs AG** will provide a platform for discussion, and a means of raising awareness among stakeholders. To do this, we plan to coordinate dialogue in the community in order to further improve and standardize the information generated by scientists making it usable by decision makers, facilitating the innovation of ethical and cost-effective testing procedures. In the short and long term, this AG wants to be a key player in a global dialog, cementing a leading role for SETAC scientists while fostering ongoing discussions about how to proceed on a scientific level to maximize gains for industry and policy makers around the world.We want to take this opportunity to invite the community to join this discussion and contribute to a better environmental and human protection without jeopardizing our need for development and industry high rate of “responsible innovation”. We believe that only an open and honest dialogue among environmental risk assessors, academia, industry, regulators and legislators coupled with shared understanding of each other needs will be able to bring the environmental protection to a good point.

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Molecular toxicology approaches to address the adverse outcome pathway for narcosis toxicity using Caenorhabditis elegans and the RTgill cell line as model systems

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Narcosis, or baseline toxicity, is a toxic mode of action predicted by QSAR analysis to occur in over 70% of industrial chemicals. However, there is currently no clear understanding of the mechanism of toxicity for these types of chemical stressors. Hypotheses include impacts on the electron transport chain, impacts on calcium signaling via the sarco/endoplasmic reticulum calcium ATPase, and an overlap with the mechanism of analgesic chemicals, which also act via a reversible and membrane-based mechanism. While our group has developed insights into potential genes and pathways of interest using computational biology approaches, we are also directly analyzing physiological responses. The objective of this project is to use *C. elegans* as a model organism for evaluating responses to narcosis stressors on a non-transcriptional level. Based on results from our transcriptomics experiments, we hypothesize that genes related to collagen structure and oxidative phosphorylation may have a role in the toxic responses elicited during narcosis. To evaluate this hypothesis, we conducted follow-up experiments to validate the role of these genes and pathways in narcotic responses. We validated gene expression of the collagen protein gene col-19 in *C. elegans* as being differentially expressed between polar and non-polar narcotics (one-way ANOVA, $p < 0.0001$). The role of this gene in narcosis toxicity responses (e.g. LC50) will be assessed using RNA interference (RNAi) approaches, where col-19 will be selectively knocked out followed by exposure to a panel of polar and non-polar narcotic chemicals. Based on results collected from a separate study in *Daphnia magna*, we are also using this approach to address the role of the *C. elegans* calcium ATPase (sca-1) by knocking down the function of this gene and comparing LC50 responses. We also directly address the impact of narcotic exposure on calcium signaling and oxidative phosphorylation using cell imaging and the Biosciences XF24 platforms respectively. These tools enable us to determine the physiological impacts of narcotic exposure. These physiological data will be linked to our transcriptomics and metabolomics data sets, as well as with parallel studies using the *in vitro* model RTgill cell line, in order to develop a robust adverse outcome pathway for narcosis toxicity and for incorporating this information into improved screening and assessment of chemical risk for this class of compounds.

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Network analysis of gene expression profiles identifies gene networks driving changes in reproduction: a case study in Daphnia exposed to different binary mixtures.

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Over the last decade, molecular technologies have evolved into robust high throughput platforms yet implementation of these technologies in ecotoxicology and risk assessments remains difficult and is limited to small datasets. As a result, knowledge of stress responses and mechanisms of toxicity across stressors and particularly mixtures remains limited. Here, we report the use of high throughput microarrays to study 48 binary mixture combinations of cyanobacterial stress and insecticide contamination to *Daphnia*. We identified gene networks that correlated significantly with changes in reproduction in *D. pulex*. These gene networks were diverse in size and function and could be represented by a small subset of 10 core or hub genes. This suggests that (1) only a limited number of genes is driving expression patterns that correlate with reproduction at the organismal level and (2) these limited subset of genes can be divided into several independent gene networks. The results highlight the potential of network analysis in combination with high throughput molecular data for environmental risk assessment.

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Developmental neurotoxicity assessment of chemical exposure in children: weight of evidence approach using in vivo behaviour, metabolomic and proteomic studies

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Worldwide, serious concern has arisen about the increased incidence of learning and developmental disorders in children. From a scientific point of view, there is no doubt that exposure to neurotoxic chemicals during early brain development can adversely affect learning and development. Various recent epidemiological studies have indicated that exposure to low doses of environmental biologically active contaminants (e.g. pesticides) during human development can have deleterious effects on cognitive development in childhood. The European commission-funded project DENAMIC "Developmental Neurotoxicity Assessment of Mixtures in Children" investigates neurotoxic effects (e.g. learning and developmental disorders) of low-concentration mixtures of pesticides and a number of common environmental pollutants in children. We focus on (subclinical) effects on learning (cognitive skills) and developmental disorders in children (e.g. ADHD, autism spectrum disorders and anxiety disorders). The aims are to develop better and sophisticated tools, procedures and testing methods to screen compounds for (developmental) neurotoxicity, and to improve our understanding of chemical exposures and the observed effects (www.denamic-project.eu). As part of the project, a new alternative assessment strategy based on a combination of *in vitro, in vivo* assays, omics, and human exposure assessment is under development in order to prioritize compounds, and to further investigate the pathways and mechanism involved in disorders and diseases. The final aim of DENAMIC is to reduce effects of environmental contamination on learning and developmental disorders in children. In the current study, a weight of evidence approach is followed to improve our understanding of the underlying molecular mechanisms of observed effects on behaviour and cognitive function of various pesticide exposure in rats using metabolomics and proteomic studies.

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Potential and Limitations of Comparative Genomics for Predicting Pharmacological Effects of Pharmaceuticals in Fish

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Identification of orthologous genes is an important step in the assessment of evolutionary conserved functions. Within the field of (eco)toxicology, information regarding orthologous relationship holds promise to predict conserved, chemical induced, mode of action (MoA) across species and thus help to guide the selection of appropriate test species for further testing. We have started to explore the potential and limitations of such an approach by analysing orthologous relationships between human drug targets (466) and corresponding proteins in more than 40 vertebrate genomes, including 13 species of fish (11 teleosts). We have previously shown that ~ 85% of the human drug targets are conserved in fish. A growing number of publications comparing pharmacological effects and therapeutic concentrations in humans to internal concentrations and pharmacological effects in fish provide support for ortholog detection as a strategy to inform extrapolations of effect data across species. It is more challenging however to identify species and taxa that are lacking orthologs and/or effects. To address this question we focused our predictions on the identification of drugs that are lacking pharmacological effects in fish. We have analysed predictions of orthologous relationships from two databases (Ensembl Compara and Inparanoid), which uses different strategies for the predictions. Only three of 976 drugs lack orthologs to all pharmacological targets in all fish studied. In the case of Amiloride, despite the absence of the sequence targets, published studies show it can induce specific, pharmacologically related effects in fish (inhibition of sodium transport), albeit at concentrations several orders of magnitude higher than in humans. This effect (in fish) is mediated via conserved off-targets. The example re-enforces the need to consider effective concentrations and off-target effects in API assessments. The results of the ortholog detection also predict that 84 drugs (46 targets) are lacking all pharmacological targets in at least one but not all species of fish. Additional data, supporting these potential gene losses were however available only for two targets. In conclusion, ortholog detection holds good promise for identification of conserved chemical MoA across species but the lack of predicted orthologous targets doesn't rule out biological effects for drugs, and thus findings from such an approach need to be interpreted with caution.

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Toxicity testing and transcriptomics with Daphnia magna and application to Danube risk assessment in SOLUTIONS
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The SOLUTIONS project aims to find new and improved tools, models, and methods to support decisions in European environmental and water policy. In addition to a wide selection of bioassays, transcriptomics assays are being performed using Daphnia. Crucially, the extensive chemical and ecological characterisation of environmental sampling sites on European rivers presents the possibility of linking chemical data to adverse ecological outcome via biological responses following the AOP paradigm Large volume solid phase extraction (LV-SPE) was used to concetrate organic extracts from 1000 litres of surface water at 25 sampling sites on the Danube during the Joint Danube Survey 3 (JDS3) project and SOLUTIONS. Acute toxicity of the Danube LV-SPE samples to Daphnia was tested experimentally by a modified version of OECD Test No. 202[3]. Neonates from sublethal exposures were collected and frozen at -80C prior to RNA preparation and transcriptomics. Transcriptomics employed a novel 8x60k *Daphnia magna* Agilent oligonucleotide microarray design. From chemical data, PAHs (benzo(g,h,i)perylene, indeno(1,2,3-c,d) pyrene and fluoranthene) and the AChE inhibiting insecticide diazinon were predicted to pose the most risk of Daphnia acute toxicity, considering all Danube sites. Daphnia toxicity determined experimentally correlated positively with predicted toxicity ($r=0.49$; P-value < 0.05), although several outliers were noted. At the Novi Sad site toxicity at the effluent inflow was significantly higher ($P< 0.05$) than immediately upstream. Daphnia transcriptomic responses to the Danube environmental extracts provide data to characterise the similarities and differences between sites of different pollution profiles. Although there was a positive correlation between Daphnia toxicity predicted from chemical concentrations and those determined experimentally, several outliers showed that additional factors influence toxicity. These are likely to include chemicals not detected or considered in the analysis, or for which there is insufficient Daphnia toxicity data, as well as interaction between components of the mixtures. Detailed transcriptomic investigation of Daphnia responses to the different environmental extracts, in combination with chemical, ecological and other bioassay data, have the potential to reveal candidate compounds associated with deviation of toxicity from that predicted by risk assessment

Challenges in Environmental Assessment of Cosmetics and Personal Care Products

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Regulation of cosmetic ingredients under REACH: Is it necessary? - A case study with 3-BC and 4-MBC
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Many active ingredients of cosmetic products can enter the environment during use and disposal. Special concern is arising from the group of commonly used UV filter substances in sunscreens and other cosmetic products since they enter surface water bodies directly via wash-off during use and without being processed within waste water treatment plants. The problem here is that the EU cosmetic regulation does not allow for higher tier testing using in vivo assays. Thus, PBT, vPvB and e.g. endocrine disruption, being the most critical hazards arising from chemicals from an environmental point of view, cannot be evaluated based on the data requirements under the cosmetic regulation. The presented case study should demonstrate, using the example of two camphor-based UV filter substances, how the identification of environmental risks and their management can be addressed under REACH. In this context a research project dealing with the relevance of potentially endocrine disrupting substances for regulation the uses and findings of 3-BC and 4-MBC in the environment were evaluated. Furthermore, a sound evaluation of the SVHC properties of both camphor substances is being performed under REACH aiming at identifying both UV filter chemicals as substances of very high concern for the environment. Finally, a risk management option analysis (RMO) is performed to identify the most adequate and effective risk reduction approach. Part of this evaluation is also a discussion of the substances within the ECHA expert groups (e.g. the Endocrine Disruptor Expert Group) and a public consultation of the outcome of the RMO analysis and the SVHC identification. This ensures a transparent process for all stakeholder groups being involved. The REACH framework provides useful tools to evaluate and to manage cosmetic ingredients of environmental relevance. Especially the option to request higher tier in vivo data under the substance evaluatiuon process within REACH, which is not possible under the EU cosmetic regulation, is of high relevance to clarify hazardous properties of very high concern (CMR, PBT, vPvB and e.g. endocrine disruption) of cosmetic ingredients. Thus, assessing cosmetic active ingredients under REACH can be an efficient approach to minimize their environmental and/or human health risks and be a potent driver for substituting critical chemicals in cosmetic consumer products.

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UV-filters and musk from Personal care products in coastal regions : Seasonal and diurnal trend in mussels and seawater.
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UV-filters and musks fragrances have come into focus because these compounds are increasingly used in sunscreen products and in many products of daily use, such as cosmetics, skin creams, plastics or varnish. These compounds have gained increasing interest due to their occurrence in the environment and to their potential to cause endocrine disruption. UV-filters have been reported in coastal regions mainly due to recreational bathing activities, including both sea water (Sankoda et al. 2015) and marine mussels (Bachelot et al. 2012). The highest concentrations of UV filters in mussels were reported for two substances, ethylhexylmethoxycinnamate (EHMC) and octocrylene (OC) and occurred in sites with a strong touristic frequentation and a geomorphological conformation of the beach closed to the wide. In this context, the objectives of this work were three folds: firstly, a monitoring of the seasonal variations and diurnal trends of these emerging contaminants was conducted during the touristic period. Secondly, the relation between the concentration in water and in mussels was studied within one day for a better understanding of the temporal trends. Finally, the contribution of WWTP releases on coastal contamination was investigated. The extraction of mussel tissues was based on a QuEChERS procedure (Picot-Groz et al. 2014). Sea water was extracted with semi-preparative extraction (SPE) method. Analysis was performed with liquid chromatography coupled to high resolution mass spectrometry (LC-MS/MS) and gas chromatography-mass spectrometry (GC-MS/MS). The developed analytical method allowed to detect target compounds at low levels under 10 ng/g in marine mussels and under 1 ng/L in sea water samples. A seasonal variation of OC and EHMC was observed with the highest concentration in mussels reported in July while the strongest touristic pressure. The diurnal trend of UV filters was similar in sea water and in mussels. These results suggest a high bioconcentration potential in mussels, and probably a metabolism ability for these substances. This is in accordance with previous findings where mussels feed with contaminated phytoplankton bioaccumulated low UV-filter concentration (Gomez et al. 2012). One UV stabilizer (UVP) and one musk (galaxolide) occurred in mussels from a non protected area, indicating other inputs than bathing. The reason evoked is the arrival of WWTP rejects that do not allow a complete elimination of these compounds.

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UV filters bioaccumulation. The need for metabolites inclusion when carrying out ERA
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Environmental Risk Assessment (ERA) considers the impact on the environment caused by substances in daily use products, pesticides, etc. In addition to ERA, it is also wise to assess the risks posed to human and animal health by chemicals which may be present in the environment and consequently may enter the food chain. After use, personal care products (PCPs), may be absorbed by the body and excreted or washed off. PCPs and their metabolites enter the aquatic environment. There, they are partially eliminated in WWTPs and released to the water bodies through the effluents [1]. On the last ten years, the concern about the potential hazardous risk associated to them and their byproducts, that can be more persistent and toxic [2], has been on the rise. In this study we investigated the bioaccumulation potential of BP3, BP1, 4HB, 4DHB, OD-PABA, OC, EHMC in wild seafood of economic significance to provide data for a reliable ERA. In Brazilian fish samples gills, muscle and liver were analysed separately to estimate accumulation distribution among tissues. Pressurized liquid extraction (PLE) with in-cell purification of the samples was applied as extraction and purification technique. Extracts were analyzed by HPLC-MS/MS [3]. 4MBC was the most frequently compound observed in Perú samples and at the highest concentration

(549 ng/g dw in *Aulacomya ater*, "choro"). In the samples from Brazil, BP1 in the three fish tissues and OC in liver and gills were the predominant and most accumulated compounds. The occurrence of the metabolites, BP1, 4HB and 4DHB in the samples suggest that metabolites also accumulate in fish. Results revealed that UV filters were detected in all samples. Some of them are endocrine disrupting compounds that may cause negative effects on the reproduction and development of these species which may lead to their extinction and/or to affect humans through ingestion. Metabolites may be both excreted and bioaccumulated in the organisms. Thus, for a reliable ERA, metabolites should be considered. \n References [1] Nieto A, Borrull F, Marcé RM, Pocurull E. J. Chromatogr. A 1216 (2009) 5619 [2] Bester K. J. Chromatogr. A 1216 (2009) 470 [3] Gago-Ferrero P, Díaz-Cruz, M.S., Barceló, D. J. Chromatogr. A 84 (2013) 93 \n *Acknowledgement* - The authors thank Generalitat de Catalunya (Consolidated Research Group "2014 SGR 418 - Water and Soil Quality Unit"). We also thank Sylvia Nogueira and Mariana Alonso for the provision of the brazilian samples.

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Eco-SUN for Eco-design of Sunscreen Using titanium dioxide Nanoparticles

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Among cosmetics and personal care products, sunscreen products are of emerging concern regarding both human and environmental health. The fate and impact of mineral nanoparticulate UV-blockers, such as TiO_2 nanomaterials, is under consideration from a regulatory perspective due to their potential impact. Once leaving the skin either through bathing or everyday usage and cleaning, the nanomaterials contained in the sunscreen can be released into rivers, lakes, sea shores, and/or sewage treatment plants. The nanomaterial behaviour, fate and impact in these different systems is largely determined by its surface properties, (e.g. the nanomaterial coating type) and lifetime. Here we present the first result of the Eco-SUN research program aimed at developing the eco-design of sunscreens through the minimization of risks associated with nanomaterials incorporated into the formulation. Different stages of the cream lifecycle are considered from its manufacture to its end of life, through its use by the consumer and its impact on the exposed environments. Reducing the potential release and / or toxicity of the nanomaterial from the cream is a decisive criterion for its eco-design. Different relevant TiO_2 UV-blockers have been selected to integrate a typical o/w formulation as case studies. The resulting sunscreen were characterised in terms of nanomaterial localisation, sun protection factor and photo-passivation. The risk for the consumer by dermal exposure was assessed using skin biopsies. Inflammation and skin penetration were evaluated. The risk for the aquatic environment directly expose was assessed both in terms of exposure and hazard. The release of nanomaterials from the sunscreen upon normal usage was studied in laboratory through simulated aging procedure. Two biological models, sea urchin and coral colonies, were selected as relevant endpoints to assess the marine ecotoxicity of the byproducts formed. Finally, the risk related to the end of life of the sunscreen through the removal with cleaning water followed by drainage to sewage treatment plants was evaluated by considering two opposite fate scenarios: (i) nanomaterial concentration in sewadge sludge later spread as fertilizer in agriculture, and (ii) nanomaterial suspension maintained in the treated water and released in river water. Thus, fate and impact in soil and river ecosystems were also studied.

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Benthic Invertebrate Exposure and Body Burden Analysis for cVMS Materials - A Probabilistic Risk Assessment Approach

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Lipophilic chemicals, such as the cyclic volatile methylsiloxanes (cVMS) octamethylcyclotetrasiloxane (D4), decamethylcyclopentasiloxane (D5), and dodecamethylcyclohexasiloxane (D6), adsorb extensively to particles and surfaces in aqueous systems, making sediments the predominate repository when performing risk assessment evaluations. A widely accepted step for estimating the possible risk posed by such chemicals to sediment-dwelling species is to compare the observed sediment concentration with either published ecotoxicity guidelines or to chronic no-observed effect concentrations (NOECs) from toxicity testing with benthic invertebrates. The objective of this work was to compare field exposure sediment and biota levels of D4, D5, and D6 to chronic benthic invertebrate NOEC values, using a probabilistic risk assessment (PRA) approach and Monte-Carlo simulations to estimate the likelihood of exposure/toxicity overlap and to analyze the uncertainties associated with the risk assessment. For the three cVMS materials, no overlap of sediment exposure concentrations and chronic NOEC values was noted, with exposure distribution probabilities ranging from 95% to 99% and with chronic NOEC extrapolated probabilities of 1 to 5%. PRA successfully described the overlap of sediment exposure and chronic benthic NOEC distribution datasets for the cVMS materials. The use of Monte-Carlo analysis yielded descriptions of the probability of overlap of exposure and chronic NOEC, i.e., a quantitative description of the risk posed by these materials to benthic invertebrate organisms/populations. Using both sediment OC-based and lipid tissue-based analyses, the cumulative probability of exceedence of chronic NOEC levels for tested benthic invertebrate species is

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Towards A New Method for Performing Environmental Risk of Complex Substances (CRANCS).

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Complex mixtures pose a significant problem for environmental risk assessors in Europe as guidance is unclear how to deal with input data. Aquatic ecotoxicity data can be generated using the Water Accommodated Fraction (WAF) method but derivation of PNECs in other compartments is not simple. Complex mixtures deposited into receiving water will differ in partitioning between the aquatic and sediment compartments, leading to a different constituent profile from the original mixture. The objective of the "Compartmentalised Risk Assessment for Natural Complex Substances" (CRANCS) project is to develop a predictive method for environmental risk assessment in aquatic medium for Complex Substances, notably essential oils. Concentrations of the constituents of known mixtures will be spiked to characterised sediment-water systems and measured concentrations of each constituent will be compared to those calculated using standard partitioning methods. Alternative partitioning calculations may be developed to quantify the partitioning of constituents between aqueous phase and sediment phase. This information will be used to define the PECaquatic and PECsediment. It is expected that the pelagic and sediment compartments will contain different mixtures (sub-mixtures) from each other and from the original complex substance. The spiked compartments will be tested for the influence these mixtures have on ecotoxicity, and a risk assessment will be carried out in both compartments: pelagic (water) *versus* benthic (sediments) at equilibrium using the PECs and acute and chronic aquatic toxicity data. The results will be compared to approaches currently used for estimating risk based on the original complex substance. The experimental phase will be performed on two complex mixtures (one prepared specifically for this study, containing not readily biodegradable, stable substances and a second one on a known NCS). The modellers will simulate the results *in silico* using the WAF mixture calculation on each sub-mixture (pelagic and sediment). An attempt will be made to calculate the sediment WAF validated by the experimental results. Ultimately it is hoped that the model can used in future risk assessments to accurately predict the ecotoxicity of NCS in both the water and sediment phases. This would significantly reduce the amount of experimentation necessary and should also be considerably more precise than the current risk assessment methods available for complex substances.

Endocrine Disruptors: Exposure, Hazard & Risk Assessment (I)

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SETAC Pellston WorkshopTM: Environmental hazard and risk assessment approaches for endocrine-active chemicals (EHRA): developing technical guidance based on case studies to support decision making.

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Suspected endocrine disrupting chemicals (EDCs) are now being evaluated by several regulatory authorities. A debate is in progress about whether or not EDCs can be adequately assessed by following the standard approach involving identification of intrinsic hazards, prediction of exposure and consequent calculation of risk, or if hazard alone should be used to decide if chemical registration can be permitted. Now that a number of mammalian, non-mammalian and *in vitro* test guidelines have been adopted by the Organisation for Economic Cooperation and Development (OECD) and the US Endocrine Disruptor Screening Program (EDSP) has generated significant data, the key challenge remains the integration of scientific evidence to support the hazard and risk assessment of chemicals with known or suspected endocrine activity. For this reason the SETAC-Pellston Workshop^{TM}Environmental Hazard and Risk Assessment Approaches for Endocrine-active Chemicals (EHRA) will be held from the 31st of January to the 5thof February 2016. Through the development of case studies it aims to develop tools that can be used by regulators and other decision makers involved in the assessment of chemicals with endocrine activity. 50 carefully selected scientists from a range of disciplines are working in case study groups each evaluating one of six chemicals known or suspected to have endocrine activity: 17 alpha ethinyloestradiol, trenbolone, perchlorate, propiconazole, vinclozolin and tributyltin. Per chemical approximately 150 publications and publicly available regulatory data have been selected for detailed evaluation. Following a Weight of Evidence assessment focused on endocrine activity, the main environmental hazards will be quantified looking at relevant taxa. Risk assessments for both endocrine-active and non-endocrine-active endpoints will be conducted. While going through this process the case study groups are asked to focus on a number of issues that cut across the different case studies and reflect the challenges that are faced when determining hazard and/or risk of endocrine active substances (EAS). The primary output of the workshop is the Synthesis Document which will be a technical, scientific guidance document primarily for regulators to support decision making in environmental hazard and risk assessment approaches for EAS. The presentation will discuss objectives, approach, output and regulatory impact of the workshop.

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Endocrine disruptors - Regulatory decision making process at ECHA's Member State Committee

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REACH is an EU Regulation that requires 'standard information requirements' to be provided by manufacturers and importers of industrial chemicals in the EU. Under dossier evaluation, carried out by ECHA, the information in registration dossier is compared with the standard information requirements found in Annexes Vi to X of REACH. Further information may be requested if data gaps relevant for the safe use of the substance were identified. On the other hand, under substance evaluation, were Member State Competent Authorities (MSCAs) carry out an evaluation of a substance prioritised based on risk concerns and listed on the Community Rolling Action Plan (CoRAP), information that goes beyond the standard information requirements of REACH can be requested. Depending on the concern, more sophisticated experimental data or information on exposure or hazard (e.g. information related to Mode of Action like in relation to endocrine disruption or genotoxicity) may be requested for the priority substances. Some tests targeted for endocrine disruption are considered non-standard information requirements (e.g the fish Sexual Development Test or the Uterotrophic Assay in rats). ECHA's Member State Committee (MSC) is responsible under REACH for resolving divergent views among Member States and ECHA as regards the information Registrants and Downstream Users are required to generate to show that their substance can be used safely without harm to man or the environment. Furthermore, MSC can identify substances of very high concern (SVHC) for instance on the basis of CMR or PBT/vPvB properties. Endocrine disruptors may be identified on a case-by-case basis as SVHCs, where there is scientific evidence of probable serious effects to human health or the environment, which gives rise to equivalent level of concern to CMR or PBT/vPvB substances under Article 57 (f) of REACH. Two case studies to exemplify MSC's considerations and decision making process will be selected: one from a substance evaluation (benzophenone (BP)) and another case study from a SVHC identification (Bis(2-ethylexyl)phthalate (DEHP)). The discussions for each case study at the MSC based on the lines of evidence will be presented explaining the resoning for the change in the proposed testing strategy for the BP subsance evaluation case and for the identification of DEHP as an SVHC with endocrine disruptive properties towards wildlife.

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Identification of environmental endocrine disrupting chemicals under REACH - Experience and challenges

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Horizontally harmonized criteria for the identification of endocrine disrupting chemicals (ED) are still missing at the EU level. While such criteria are urgently needed to regulate ED substances under the plant protection product and biocidal product regulation (PPPR and BPR), under REACH the identification and regulation of ED is already possible without the lack of harmonized criteria. This is due to the fact that under REACH the identification of an ED can be treated separately from the following risk management, which is not the case for plant protection products and biocides. The work presented here will reflect our experience with the identification of ED in the environment under REACH and tries to work out why from an environmental point of view the identification of ED differs from the approach used for human health risk assessment. The resulting problems concerning the design of harmonized identification criteria are also addressed. The differences and the commonalities of the PPPR and BPR regarding the identification and its consequences are summarised. The process under REACH is discussed in detail and using examples from already identified ED it is presented how the requirements of Art. 57 (f) of the REACH regulation can be fulfilled without having horizontal criteria for ED identification. It is generally agreed that there are many uncertainties in carrying out a risk assessment of ED. Many are not specific to EDs but some factors may be particularly relevant to some ED. Endocrine Disruptors may cause adverse effects in a variety of species including very different taxa. With respect to this we have the following position: It is at the moment impossible to identify, which species are sufficiently representatives for wildlife with regard to endocrine effects. The available test methods are very limited and especially with regard to invertebrates do not cover sensitive taxa and life stages. It might be possible to overcome these shortcomings in future but based on the already available indications of harmful effects in the environment, it seems not to be adequate to await this progress. Thus, we think that the ED identification should be based on WHO/IPCS definition and should consider that uncertainties are high for ED with regard to environment. This should result in a hazard based regulation but regulatory consequences for different legislation need to be taken into account (i.e. exemptions needed for plant protection products and biocides).

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What, oh what, is the mode of action? Use of the acute to chronic ratio in a weight of evidence for endocrine activity

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Extensive discussion is occurring in the European Union and internationally on how to identify and potentially categorize endocrine disruptors (ED). There is general agreement that the evaluation of ED should be based on the WHO/IPCS (2002) definition. According to WHO/IPCS: *"An endocrine disruptor is an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse effects in an intact organism, or its progeny, or (sub)populations*." This definition embodies the key elements of adversity, endocrine mode of action (MoA), and a causal link between endocrine activity and adverse effect. The mere existence of an endocrine mode of action (*i.e*., endocrine activity) is not a harmful property, a type of toxic effect, or a hazard. Rather, endocrine activity, if causally linked to an adverse outcome, can potentially lead to a hazard to health or to the environment, particularly after long-term exposure. Defining that there is a causal link between an indication of activity and an adverse effect, particularly one that would be the lead or primary toxic effect most relevant for risk assessment is important. Identifying the causal link can be difficult in studies with many apical endpoints. The acute to chronic ratio (ACR) can be informative in situations where there are in vitro indications of activity but the weight of evidence for an in vivo causal link to an adverse outcome is not clear. The extensive database for BPA will be used to illustrate a robust weight of evidence process for a substance with weak in vitro estrogenic activity and in vivo biomarker responses. Although a weak estrogenic activity is apparent for BPA among various fish species, the profile of adverse effects that have been measured in BPA-exposed aquatic vertebrates and are used in ecological risk assessments (*i.e*., effects on survival, growth, development, and reproduction) are not all definitively linked to a weak estrogenic mode of action. The ACR for BPA, in comparison with substances with hormonal modes of action, will be used to help clarify the differences between substances that do have chronic hormonal toxicities and those that might be responding to general, systemic toxicity. The orders of magnitude difference in the ACR for BPA, compared to substances such as estradiol and ethynl estradiol, suggest that BPA's lead mode of action is related to non-specific, systemic toxicity rather than an endocrine specific response.

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Opportunities and challenges in using rodent data to evaluate ecological hazard of potential endocrine active substances

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Inter-species extrapolation of toxicity data is a substantial challenge for environmental risk assessment. Due to practical limitation in the number of *in vivo* assays that can be conducted, and the desire to reduce animal testing, regulatory decisions commonly rely on few whole-animal studies. Additionally, ecotoxicological hazard assessments most often rely solely on aquatic species (fish and amphibians). This has led some to consider the use of rodent data to evaluate endocrine active compounds for terrestrial assessment. While this has advantages, including a direct comparison to terrestrial mammalian species, evaluating such data in the context of endocrine disruption encompasses many challenges and unknowns. For example, macroscopic (e.g. routes of exposure and life histories) and microscopic (cellular receptors and pharmacokinetics) differences between rodents and other terrestrial vertebrates (including avian and reptilian species) are poorly understood. Additionally, field-derived metabolic rates have shown to vary widely between vertebrate taxonomic groups (up to five orders of magnitude). Energetics will influence behavior, biochemical pathways and food intake, all of which may alter hazard potential. Furthermore, rodents in a laboratory will not be subject to the same seasonal influences, which greatly affect the endocrine system, and therefore may not exhibit similar exposure effects/sensitivities. Finally, while rodent models and human health are focused on hazards to individuals, investigating endocrine disruption in the environment requires evaluating adverse effects at the population level. Here we further discuss some challenges and opportunities in extrapolating rodent data to adverse environmental effects at the population level. This discussion will inform future research and regulatory action when assessing ecological hazard assessment. While rodent data may be reasonable to evaluate endocrine disruption for terrestrial species, it is not universal, and factors such as exposure route, pharmacokinetics, and taxonomic differences in metabolic pathways must be considered when determining adverse population level effects in the environment.

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Quality Standards for Nonylphenol and Octylphenol in sediment based on ecotoxicological studies
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Nonylphenol (NP) and octylphenol (OP) are degradation products of alkylphenolethoxylates (APE), non-ionic surfactants employed for more than 40 years, which elicit endocrine disrupting effects on aquatic organisms. For this reason, since 2001, they have been included in the list of priority hazardous substances of the Water Framework Directive. Quality standards (QS) for different environmental compartments were set out, and NP, OP and APE have been subject to EU restrictive regulation on marketing and use since 2003. The significant decrease in NP and OP concentrations in water in the last years demonstrates the effectiveness of the control measures that the European Union put in place to control these priority hazardous substances. However NP and OP concentrations in monitored sediments are often above QS for sediments raising doubts about the reliability of these QSs. In the framework of a European initiative for the harmonization and derivation of Environmental Quality Standards, Italy and Denmark collaborated to derive a robust and reliable QSsed for these two priority substances. Toxicological tests with *Chironomus riparius, Lumbriculus variegatus and Hyalella Azteca* were carried out according to OECD and USEPA guidelines for both NP and OP. The test results and literature data were combined and a QSsed for NP and for OP based on ecotoxicological studies was derived according to the Technical Guidance Document on deriving EQS (CIS-WFD Guidance n. 27). The estimated QSs for sediment, normalised to organic carbon content, were 37 and 25 mg/kg OC respectively for OP and for NP. These QSs for sediment, based on ecotoxicological studies, are about 100 higher the previously derived QSs which were calculated by Equilibrium Partition (EqP) approach.

Advancements in life cycle impact assessment method development (I)

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A first step towards the consideration of habitat fragmentation in LCA
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Fragmentation is commonly defined as a process in which "a large expanse of habitat is transformed into a number of smaller patches of smaller total area, isolated from each other by a matrix of habitats unlike the original". Alongside climate change or pollution, habitat change and fragmentation was identified by the Millennium Ecosystem Assessment as one of the five direct drivers of biodiversity loss. Given this fact, it seems to be of paramount importance to include this driver in LCA methods. To this end, the last theoretical frameworks of land-use impact chains proposed to include fragmentation as a human intervention leading to biodiversity loss. Nonetheless, the UNEP-SETAC land-use group recently pointed out that habitat composition and configuration, including fragmentation, seemed to be under-represented in impact assessment models and should be considered, particularly when dealing with Species Area Relationships (SAR). A literature review on the ecology field was performed in order to define fragmentation and its relating environmental mechanism and to identify quantitative approaches that attempt to consider the impacts of fragmentation on biodiversity loss. Among these approaches, recent work on adapting the SAR in order to include fragmentation effects along with area loss seems to be promising. The Species Fragmented-Area Relationship (SFAR) uses the metapopulation capacity λ to describe the degree of fragmentation of a landscape. This work proposes to adopt this method to consider fragmentation in LCA. First, we developed a routine procedure to derive quantitative values of λ for different landscapes. Second, we applied the methodology to two contrasted Amazonian landscapes to test its feasibility and interest. The results show that fragmentation has important effects on spatial configuration and should be included in LCIA methods. However, one limitation of the SFAR is its relatively recent methodological development, and the fact that so far, it has only been tested in an Amazonian ecoregion for birds, while a larger spatial and taxa coverage for a practical use in LCA is required. Therefore, it is important to assess the validity of the method in other contexts. In conclusion, this exploratory work aimed at assessing the feasibility of including one of the most comprehensive and operational ecological models for fragmentation into LCA. The next step will be to derive CFs for land-use from the SFAR in order to conclude on its relevance.

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Advancing marine impact assessment within life cycle assessment (LCA)
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Assessment of ecosystem damage in life cycle assessment (LCA) has greatly progressed over recent years. However, marine ecosystems remain heavily underrepresented, despite their importance for anthropogenic activities, both as a source of resources and receptor of waste, meaning that impacts on marine ecosystems are often assumed negligible. We identified seven major drivers for marine biodiversity loss: climate change, seabed damage, ocean acidification, marine eutrophication, overexploitation, invasive species and plastic waste. Characterization factors associated to some of these impact categories have already been developed. However, (spatial) detail and (geographical and taxonomic) coverage remain limited. For other impact categories, such as marine plastic debris, no LCA approaches exist yet. Furthermore, others have only case–study-based LCA approaches, for example invasive species. For all of the seven drivers, models predicting the distribution, intensity and damage of anthropogenic interventions, both temporally and spatially, are available. We therefore review possible quantitative approaches for including, or refining the representation of, these seven impact categories in LCA. We find that it is possible to derive impact indicators for all considered drivers. However, the levels of detail, coverage and uncertainty of the factors may still vary. In addition, fate and exposure factors are more readily developable than effect factors. For plastic debris, for example, models are available that describe the transport of plastic waste from the land to the ocean and the subsequent accumulation procedure within the ocean itself. These approaches can be translated into a fate factor by assigning to each country the fraction of plastic that is ending up in one of the different accumulation zones in the ocean. Models to estimate the effects on biodiversity are less developed and variation in model sophistication is large. Whilst well-established species sensitivity distributions can, for example, be used for marine eutrophication, ocean acidification and particle sedimentation, models for indicating the effect of plastic debris on marine biodiversity are completely lacking. We recommend adapting available quantitative approaches to generate new and refine existing fate models, and to further development of effect factors based on species richness loss, analogous to potentially disappeared fractions of species.

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Accounting for biodiversity impacts of seabed damage in life cycle assessment
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Traditionally life cycle assessment (LCA) has mostly focused on estimating impacts on terrestrial and freshwater ecosystems. Due to limited coverage of marine-focused operational life cycle impact assessment (LCIA) methodologies, impacts on marine ecosystems are often assumed negligible. Given the importance of the marine environment for human society, for example through provision of mineral and fish resources, and a desire for LCA to be a comprehensive environmental assessment tool, development of novel marine-focused LCIA methodologies is essential. Even though it is a predominant driver of biodiversity loss in marine ecosystems, one of the neglected categories is seabed damage. Some first steps in methodological development for this impact category have been undertaken in the LCA community. However, limitations remained, particularly because of limited consideration for biodiversity-specific impacts. We established a generalised impact pathway triggered by seabed damaging activities. This impact pathway applies for three main modes of impact: 1) abrasion (e.g. benthic trawl fisheries), 2) smothering (e.g. deposition of dredged material, cutting pile formation due to offshore oil and gas production) and 3) extraction (e.g. aggregate dredging). We developed characterisation factors (CFs) using a modelling approach analagous to that developed for the assessment of land use impacts. As such, the overall impact of seabed damage is a function of disturbance characteristics (scale and intensity), initial response of the benthic community, duration of the anthropogenic activity and subsequent physical and ecological recovery of the seabed. Our CFs allow for quantification of temporary impacts in terms of potentially disappeared fraction of species (PDF) due to occupation ($PDF.m^{-2}.yr^{-1}$) and transformation ($PDF.m^{-2}$) of the seabed. Our CFs also allow for quantification of permanent impacts ($PDF.m^{-2}$) if complete recovery is not

expected. The CFs are mode of impact specific and spatially differentiated according to the large marine ecosystem (LME) biogeographical system and seabed substrate. Given that most anthropogenic seabed damage occurs within continental shelf areas, our developed methodology is applicable for the global extent of the 66 LME units, which covers all continental shelf areas globally. For the first time we are able to account for biodiversity impacts of seabed damaging activities within LCA on a global scale (LME) and at endpoint level.

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Habitat suitability: water use impact assessment for ecosystems beyond counting species
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Assessment of water-use impacts on ecosystem quality in LCA is a relatively recent research domain and still undergoing continuous development. The most recent approaches develop characterization factors by differentiating *fate*, *exposure,* and *effect factors* for distinct water sources and ecosystems. Even though, there is no consensus about what are the midpoint and endpoint indicators to be chosen to better represent impacts on the *Ecosystem Quality* area of protection. Current methods are based on describing ecosystem quality by means of biodiversity loss indicators. If choosing such type of endpoint unit allows a straightforward representation of results, they still carry a lot of uncertainty due to difficulties in isolating direct causal relationships between fate and effect factors, and between midpoint and endpoint indicators. Moreover, most of the methods face problems of data availability, along with a limited spatial coverage. This raises the question whether species loss is suitable and representative for describing freshwater ecosystem quality in LCA. Adopting species-loss-based indicators for water-use impacts on ecosystems, conceptually conflicts with the natural behaviour of aquatic ecosystems, where species disappearance usually occurs in case of extreme or long term habitat alteration instead of marginal changes. Therefore, the dynamics of stressors should be characterized in order to assess impacts of water use. Eco-hydrological methods identify variations of ecologically significant parameters of flow regimes related to ecological responses in freshwater habitats. Microhabitat simulation methods are used to build habitat suitability curves for single or grouped fish species based on these variations. This study is aimed at developing an effect factor based on eco-hydrological approaches. We present first results showing the application in a selected case study in France. Going beyond indicators of species richness allows to isolate the cause-effect chain between water deprivation and ecological response from other stressors. The potential change in habitat suitability could be used as a proxy to indicate the response to habitat change for target species. Building an impact assessment method on significant relationships between freshwater ecosystems and environmental flows may ultimately require a translation into biodiversity metrics to allow a straightforward comparison and aggregation of results with other impact categories and pathways.

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Natural Resources as an Area of Protection in LCA - outcomes of the discussion by the working group on resources within the UNEP-SETAC Life Cycle Initiative
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The topic of resources as an area of protection (AoP) in life cycle assessment (LCA) is being discussed within an expert group under the umbrella of the Life Cycle Initiative by the United Nations Environment Programme (UNEP) and the Society for Environmental Toxicology and Chemistry (SETAC). The AoP 'Natural Resources' is neither well defined nor agreed upon. Furthermore, there is currently no life cycle impact assessment (LCIA) method available that is able to consistently assess impacts at midpoint and endpoint level across different resource categories (minerals/metals and fossil fuels, water, land/soil, biotic resources like wild plants and animals). Definitions and categorizations of natural resources differ and there is no agreement on what methods should be considered midpoint or endpoint methods because there is no agreement (at midpoint and endpoint) on what impact should be assessed (is it reduced availability, is it depletion, is it increased energy use or costs due to future resource extraction, etc.?). The merit of this working group is the broad analysis of available methods considering different resources and their integrated discussion according to the methods' underlying principles (e.g. use-to-availability ratios, backup technology approaches, etc.). This is the basis on which recommendations for best practice with existing methods and indications for further research and development will be given. At the time of the SETAC 2016 conference, the group should have these recommendations ready.

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Interpretation of LCA results: significant issues also informed by the environmental footprints pilots
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According to ISO 14040 interpretation is the phase of LCA in which the findings from the inventory analysis and the impact assessment are considered together; it should deliver results that are consistent with the defined goal and scope and which reach conclusions, explain limitations and provide recommendations. ISO 14044 further specifies that interpretation comprises the following elements: i) identification of the significant issues based on the results of the LCI and LCIA phases of LCA; ii) an evaluation that considers completeness, sensitivity and consistency checks; iii) conclusions, limitations, and recommendations. The Product Environmental Footprint (PEF) Guide [1] explains that interpretation of the results of a PEF study serves two purposes: i) to ensure that the performance of the PEF model corresponds to the goals and quality requirements of the study; in this sense, PEF interpretation may inform iterative improvements of the PEF model until all goals and requirements are met; and ii) to derive robust conclusions and recommendations from the analysis, for example in support of environmental improvements. Within the work done in support to PEF/OEF, the JRC has perfomed some studies and some tests in order to identify the most relevant issues to be considered in the interpretation phase. The final aim of this work is to gain insights on cross-cutting issues affecting all the phases of a life cycle study and to provide guidance and support to practitioners and researchers in the intepretation phase. The presentation will show and discuss the results of some preliminary tests done within this framework including 1. the influence to final results of the selection and the modeling assumptions in secondary inventory dataset; 2. sensitivity analysis of impact assessment modeling; 3. sensitivity analysis of normalisation, including uncertaitnty analysis; and 4. sensitivity analysis of weighting.

Passive sampling of organic micropollutants and toxicity assessment: opportunities, challenges and innovations (I)

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Detecting POPs profiles across the Atlantic Ocean using polyethlyene samplers
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Little is known of the distribution of persistent organic pollutants (POPs) in the deep ocean. Polyethylene passive samplers were used to detect the vertical distribution of truly dissolved POPs at two sites in the Atlantic Ocean. Samplers were deployed at five depths covering 26-2535 m in the northern Atlantic and Tropical Atlantic, in approximately one year deployments. Samplers of different thickness were used to determine the state of equilibrium POPs reached in the passive samplers. Comparable sampling rates were obtained from model derived results (5 & 4 L/day) and performance reference compounds derived results (8.7 & 4.9 L/day). Concentrations of POPs detected in the North Atlantic near the surface (e.g. $\sum_{14}$PCB: 0.84 pg L^{-1}) were similar to previous measurements. Currents seemed more important in moving POPs to deeper water masses than the biological pump. The ratio of PCB concentrations in near surface waters (excluding PCB-28) between the two sites was inversely correlated with congeners' sub-cooled liquid vapour pressure, in support of the latitudinal fractionation. The results presented here implied a significant amount of HCB is stored in the Atlantic Ocean (4.8-26 % of the global HCB environmental burdens), contrasting traditional beliefs that POPs do not reach the deep ocean.

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Vertical distribution of organic contaminants in harbour waters assessed by passive sampling
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Coastal and estuarine areas receive contaminants from wastewater discharges and littoral activities such as boating, sea trade and fisheries as well as from upstream inputs through riverine discharges and soil washout. Assessing the distribution of these contaminants in water bodies provides insights for the understanding of their sources and fate, hence allowing a better management of our waters. In particular, a vertical profile of pollutants can indicate sources originating from the water

surface, freshwater-derived material if correlated to salinity or surface processes such as photodegradation or evaporation at the air-sea interface. For this purpose, passive sampling is a method of choice for the monitoring of moderately hydrophobic organic contaminants at marine trace levels as they are able to concentrate the analytes in-situ, thus providing limits of detection that can be as low as the $pg.L^{-1}$. In this study, vertical distributions of selected organic contaminants were assessed in an English marina from the surface to more than 3 m depth. Polyethylene strips were held vertically and immersed for 18 days from floating pontoons. Following deployment, the strips were sliced to provide 46 depth related sub-samples. A section of approximatively 1 cm at the surface allowed profiling of the sea-surface microlayer. Time-weighted average concentrations were calculated for Polycyclic Aromatic Hydrocarbons (PAH) by using passive sampling and the performance and reference compound approach. As an example, results revealed a surface enrichment for phenanthrene and chrysene with a 5-fold increase in the first 50 cm, whilst fluoranthene and pyrene exhibited decreasing concentrations closer to the surface. Source tracking of the PAHs indicated an oil-derived contamination at the surface with enhanced combustion derived PAH concentrations at lower depths. Measurements were further extended to selected antifouling compounds, organophosphorous flame retardants and personal care products. The vertical profiles of detected compounds exhibited comparable distributions to the model PAH's which aided in interpretation of their relative sources and fates. This study demonstrates that passive sampling can provide a cost-effective and novel monitoring tool to investigate the sources and fate of contaminants whilst providing important information on compound dependent vertical profiling in contaminated waters.

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Two activities on passive sampling in water and sediment in the North Atlantic within the OSPAR/ICES community

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OSPAR Convention is the current framework for international cooperation on environmental protection in the North-East Atlantic and has installed a programme for baseline monitoring of environmental status, covering both the spatial distribution of contaminants and temporal trends. Generally, OSPAR countries monitor chemical contaminants in sediment and/or biota. Both matrices suffer from compositional heterogeneity throughout the OSPAR. To reduce sample varibility OSPAR is considering the inclusion of passive sampling in its monitoring programmes, and a number of preparatory actions on passives sampling have been conducted within the OSPAR/ICES scientific community, including the sampling of water as well as sediment. In 2006, the **OSPAR/ICES Passive Sampling Trial Survey** (PSTS) was organised with 13 laboratories participating and samples from 30 stations distributed across the ICES area applying polymer sheet samplers and bottles with 10 μm silicon polymer coatings for sediment equilibrations. Participants exposed sheet samplers were exposed to water at each of the selected stations equilibrated sedments in the laboratory. Aanalysis of duplicate samplers (sheets and bottles)by the participants and the central laboratory gave infomation on the between laboaratory variability The data produced by the central laboratory for all station allowedto evaluate the data in spatial perspective as well as over matrices. In another projekct (ICON/2008) sediment was collected at 13 stations between Iceland and the Mediterranean and equilibrated sheet samplers for 6 months . Sheet were anaysed for PRCs, PCBs, PAHs, PBDEs, OCPs, alkylphospahates, and dioxins and dibenzofurans. Passive sampling proved to be extremely sensitive and was able to detect many compounds that were not detected by whole sediment analysis. The presentation will demonstrate the power of passive sampling to reveal spatial distributions over large areas.

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Advancing in situ passive sampling techniques to quantify chemical activity gradients across the sediment-water and benthic boundary interfaces in large water bodies

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Passive sampling is increasingly recognized as a superior monitoring tool for nonpolar organic chemicals in water and sediment. Beyond monitoring, passive sampling offers opportunities to explore contaminant transport mechanisms in large water bodies; and the potential of these oppourtinities have yet to be fully exploited. Recently developed sediment porewater profilers were equipped with both μm-thin low-density polyethylene (LDPE) films and silicone (PDMS) membranes as passive samplers for polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs), offering a 2.5 cm vertical resolution of contaminant concentrations within and across the sediment-water interface and the benthic boundary layer. Passive samplers of various thicknesses were used in order to assess whether chemical equilibrium was reached during the 2 and 5 months exposure time. Passive samplers were also exposed in the water column above the sediment porewater profilers. Parallel deployment of different passive sampling materials proved to be a simple and effective tool to validate obtained passvie sampling data. Passive sampling across the sediment-water interface using the newly developed sediment porewater profiler in combination with passive sampler deployment in the water column facilitated a vertical mapping of HOC contamination and allowed a thermodynamically based assessment of contaminant fluxes.

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Determining the release of hydrophobic organic contaminants from sediment by in-situ benthic flow-through flux chambers

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Contaminated sediment may act as a source of hydrophobic organic contaminats (HOCs) to water, such as in the Baltic Sea where high chemical activity ratios between pore water and bottom water for dioxins were observed, demonstrating a potential release of dioxins from sediment to the water column. Quantification of the sediment-water flux of HOCs is important to enable assessment of the risk associated with contaminated sediment. Currently used methods to estimate the flux of HOCs from sediment to water are either indirect or direct by the use of *in-situ* flux chambers. Existing *in-situ* flux chambers are closed with no water flow through the chamber. Oxygen is therefore consumed during the deployment and benthic organisms are severely disturbed. This is a drawback of closed chambers since bioturbation may have a significant impact on the sediment-water flux. This study presents a novel *in-situ* benthic flow-through flux chamber for determination of the release of HOCs from sediment to water. The water in the chamber is kept at ambient oxygen concentration by pumping of bottom water through the chamber. The measured flux thereby includes effects due to bioturbation. Water is pumped through the chamber to oxygenate the water inside the chamber, and passes first a sorbent at the inlet to strip it of any contaminants. At the chamber outlet, a second sorbent captures contaminants that were released from the sediment during deployment. Three benthic flow-through flux chambers were deployed for 3 days in a contaminated Baltic Sea Bay (south of Stockholm) for *in-situ* testing. The flux of PAHs and PCBs [$ng\ m^{-2}d^{-1}$] was determined from the mass of compound quantified in the outlet sorbent, the area of the benthic chamber and the deployment period. 15 PAHs and 7 PCBs were analysed. Closed flux chambers were deployed in parallell and passive samplers were used to collect the freely dissolved fraction of PCBs and PAHs in bottom water and porewater (work in progress). The flux individual PAHs and PCB congeners from sediment to bottom water ranged between 60-2300 [$ng\ m^{-2}d^{-1}$] and 6-170 [$ng\ m^{-2}d^{-1}$], respectively. The low variance among the 3 replicate flux measurements supports the robustness of the *in-situ* flux measurements using the novel chamber design. The results will be further compared to flux estimates calculated from chemical activity ratios (pore water and bottom water) and with flux measurements from closed chambers deployed in parallell.

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Application of POCIS and new mixed polymer passive sampler for monitoring organic contaminants in the river Saar and the outflow of a wastewater treatment plant

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A broad spectrum of organic contaminants is released into aquatic environments and affects the water quality. For monitoring of these aquatic contaminants, passive sampling is a promising tool with a number of advantages over grab sampling. The objectives of this study were (1) to determine the dissolved concentrations of organic contaminant in the river Saar and effluent of a wastewater treatment plant (WWTP) which discharges into the river and (2) to compare the performance of two types of passive samplers. Two passive sampler types were deployed over five weeks (May-June 2015) at three locations along the river Saar, at the confluence of the rivers Saar and Mosel and in a WWTP effluent discharging into the river. One sampler was the Polar Organic Chemical Integrative Sampler (POCIS), where Oasis HLB was sandwiched between two polyethersulfone (PES) membranes. The second sampler was a mixed polymer sampler (MPS) made of Oasis HLB embedded in polydimethylsiloxane (PDMS) disks and enclosed in copper mesh to limit biofouling. PDMS is widely used for sampling neutral hydrophobic organic compounds in water due to its reversible sorption and high internal permeability. Compared to PDMS alone, the MPS has an increased affinity towards a wide range of polar contaminants with the added advantage that it makes handling of the powdery Oasis HLB easier. After five weeks deployment, targeted 46 organic contaminants of diverse log K_{OW} -0.03 for atenolol to 6.25 for miconazole were analyzed directly via LC-MS/MS. The measured sampler concentrations were calculated back to time-weighted average water concentration using sampling rates for each compound which were determined in the separate experiment. Both sampler types performed well and could detect a range of organic contaminants. Low concentrations (ng to sub μg per litre) of 27 compounds were detected in both the POCIS and MPS extracts. This includes 6 compounds from the WFD priority list, which were detected all sampling sites. For the POCIS, some of the compounds accumulated significantly in the PES membranes, which complicates interpretation of the results. The polymer block nature of the MPS made this particularly robust to handle during deployment and extraction. Future efforts are concentrated on applying the mixed polymer as a passive dosing phase, with a view to directly integrating passive sampling and dosing for measuring environmental mixture toxicity for polar and non-polar organic compounds.

Pushing nanoparticle studies to the limit - working at environmentally relevant concentrations and with complex matrices (II)

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Release of TiO2-(NP) from construction landfills

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After their use, a large fraction of nanomaterials (NMs) will be deposited in landfills, which are considered as terminal sinks for the NMs. However, experimental data and field measurements supporting this assumption are mostly lacking. Production volumes of NMs are still limited, but TiO_2 particles are used in large quantities mainly as white pigments since many years. We, thus, used TiO_2 particles as a proxy for other NMs, which may become relevant in the future, and investigated the release of TiO_2 particles from a landfill dominated by construction materials. The pH and the temperature of the oxygen saturated runoff were ~ 7 mg/l and 12 °C. Average discharge rates were a few m^3/d but increased to more than 100 m^3/d after heavy rain events. The total suspended solids (TSS) were mostly between 2 – 20 mg/l. One liter of runoff was filtered through 0.2 μm polyacetate filters and acid digested using a microwave assisted acid digestion system. The digest was diluted 1:50 with DDI water and Ti was measured on an ICP-OES. The Ti concentrations were ~10 μg/l, expect after one heavy rain event, when more than 150 μg/L Ti was measured in the runoff. This exceptionally high Ti concentration was paralleled with TSS content of more than 70 mg/l. For electron microscopy analysis, runoff was either filtered (0.4 μm) or the supernatant of centrifuged samples (5 min, 700 g) was directly centrifuged on TEM grids. About 1% of all particles (> 10'000) detected using automated electron microscopy was identified as TiO_2 particles. The particle number of all particles and also of the TiO_2 particles showed an exponential increase with decreasing particle diameter down to 0.5 μm, corresponding to the detection limit resulting from the applied microscope settings. More detailed analysis of individual particles from the supernatant in the electron microscope revealed individual TiO_2 particles in the nano-range. Single particle ICP-MS analysis revealed a total number concentration of TiO_2 particles (< 500 nm) in the order of 10^5 # / ml. These results indicate that individual TiO_2 particles are released from landfills. Although the amount of TiO_2 particles released from landfills is currently orders of magnitudes smaller than the natural background of TiO_2 in surface waters, the predicted increase in the use of NMs in building materials, consumer products, electronic devices etc. calls for a more detailed analysis of the release mechanisms of NMs from landfill sites.

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Varieties of nano-textile exposure: comprehensive sequential aging of fabrics to sunlight, washing and landfilling

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The potential for nanomaterials to be released from consumer goods is not in itself a new topic, but the basis of scientific understanding of released particles and the transformations they may undergo during the products life cycle (e.g. during storage, use and disposal) is often hampered by the narrow scope of many research endeavors in terms of both the breadth of variables studied and the completeness of characterization using multiple analytical methods. Additionally, studies of sequential aging of products representing multiple stages of the life cycle are scarce. In order to bridge these gaps for the release of nanomaterials from textiles, we conducted a comprehensive suite of studies which allowed us to suggest some overarching themes for finding important mechanisms and parameters for particle transformations when still adhered to the fabric or when released. Laboratory prepared nano-enhanced fabrics were subjected to sequential combinations of sunlight irradiation, washing (in seven different detergent formulas). Characterization is divided into two groups: analysis of solutions (washing and landfill leachate) and analysis of the NP fraction that remained on the fabric. Analytical techniques included single particle spICP-MS and TEM of the released particles, fabric digestion and total metal analysis after each exposure scenario, and both SEM and XANES analysis of the textiles for the visual integrity and speciation of Ag, respectively. Some broad generalizations suggest that sunlight irradiation hinders further speciation upon washing (as suggested by XANES). More Ag is released from fabrics than Au suggesting additional chemical influences and while a size effect may also be in play, the capping agent plays the largest role. However, when release does occur, a large proportion of particles are released into the wash water with little to no alteration of the size compared to the primary particle size (as determined by sp-ICP-MS). As seen in our previous work, detergent chemistry plays a significant role in concentrations of Ag released, where those containing oxidizing agents clearly assist in release of Ag particles (but not Au NPs, again inferring the additional chemical reactions specific to Ag). This undertaking of a large matrix of variables makes us more confident to make more overarching characterizations since trends can be monitored across more variables than in any other nano-composite release study to date.

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Fate and Bioaccumulation of Nanosilver in a Lake Ecosystem

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There is potential for the release of AgNP into the aquatic environment through discharges of municipal wastewater. AgNPs undergo dissolution in aerobic environments to release silver ion, which is toxic and has potential for bioaccumulation. The stability and transformations of AgNPs in the aquatic environment are affected by environmental factors, so the fate of AgNPs are expected to vary greatly in natural aquatic ecosystems. We added 15 kg of PVP capped AgNPs over two field seasons to a natural lake ecosystem at the Experimental Lakes Area in Canada. Stock suspensions were prepared from powdered AgNP (PVP capped; 30-80 nm) and these stock suspensions were added to Lake 222 (~600 million litre volume, 6 m maximum depth) at a rate of ~500 g per week, for a total addition of ~9 kg over the first field season (June-October, 2014) and ~6 kg over the second field season (May-August, 2015). Total Ag in the water column of L222 increased over time during both Year 1 and Year 2 of AgNP additions. Concentrations of total Ag immediately adjacent to the site of addition were >200 μg/L, but were in the range of ~5 μg/L in the epilimnion at sites elsewhere in the lake. Analysis by spICP-MS indicated that the size distribution of AgNPs remained similar to the stock suspension added to the lake, although larger particles >200 nm present in the stock were not present in the water column. The concentrations of dissolved Ag were very low (i.e.+to dissolved organic matter. Despite the environmentally relevant (i.e. low ppb) concentrations of Ag in the water and seston, there was bioaccumulation of total Ag in biota sampled throughout the lake. The concentrations of total Ag in the liver tissue of northern pike at the top of the food chain at the end of Year 2 of additions was >2 mg/kg; although the concentrations in other tissues were an order of magnitude lower. The concentrations of total Ag in liver tissues from yellow perch were approximately 25% of the concentrations in pike liver. Overall, these data indicate that AgNPs were relatively stable and widely distributed in the lake. The Ag was readily bioaccumulated in aquatic organisms. There is a possibility that biomagnification is occurring throughout the lake food web, but this requires further investigation to confirm the observation.

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Can we find nanoparticles in the Dutch environment?

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Different types of water in The Netherlands were probed for the presence of several inorganic (Ag, Au, TiO_2) and organic nanoparticles (8 fullerenes) using ICP-MS, Orbitrap-HRMS, field flow fractionation, SEM and EDX. Drinking water abstraction sites varying from dune and bank filtrates, surface waters to ground waters were analysed. Furthermore influent, effluent and sludge of sewage treatment plants, and surface waters near airports and harbours were analysed. Air samples from Rotterdam (city) and Cabauw (village) were analysed for a broad set of metals known to be used as nanomaterials in the automobile industry (Mo, Ti, Fe, Cu, Ag, Ce, W, Pd, Pt, Rh, Zn, Si and B) and also for fullerenes. The data obtained were compared to predicted concentrations in literature. In STPs silver is found in influent (1 - 5 ug/L) and effluent (0.5 - 2 ug/L). In sludge silver was found in concentrations up to 0.3 mg/kg as predicted, SEM and EDX could confirm the presence of silver nanoparticles. Gold is found in both influent (2 - 6 ug/L) and effluent (1.5 - 3 ug/L). SEM also confirmed the presence of gold nanoparticles in the sludge. TiO_2 was detected in all STP influents (25 - 110 ug/L) and effluent (1 - 4 ug/L). All STPs remove about 90% of Ti. C_{60} removal efficiency was between 90 and 99%. Up to 0.2 ng/L of C_{60} was detected in effluent. In influent the concentrations varied between 2 - 19 ng/L. In the remaining water samples, silver was only found in one groundwater sample. Amounts of gold were found in three drinking water winning sites up to 0.25

μg/L. Titanium was found in most surface waters that are used for drinking water production. None of the fullerenes were detected in the other aqueous samples. In air, concentrations of nano-sized metals (PM 0.1) increase with traffic density, however only a few mass percent is present as nanoparticles. C_{60} was found at one location (5 pg/m^3).

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Innovative combination of spectroscopic techniques to reveal nanoparticle fate in a crop plant

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Nanotechnologies are developing fast and in a number of application fields like medicine, sustainable development or plant protection products. These new applications also imply an increased dissemination of nanoparticles (NPs) in the environment. Still, their fate is largely unknown mainly because of the lack of adapted techniques to assess their behavior in complex matrixes (soil, plant or animal). In this study, we report the use of complementary spectroscopic techniques to determine the fate of two different NPs (TiO_2 and Ag) in a crop plant: lettuce (*Lactuca sativa*). The behavior of NPs was elucidated by studying their distribution by micro-X-ray fluorescence (μXRF), their *in situ* speciation by X-ray absorption spectroscopy with a micro-focused beam (μXAS) or in full-field mode and their local quantification by micro-particle induced X-ray emission (μPIXE) and Rutherford backscattering spectroscopy (RBS). After a 7-day exposure, preliminary ICP-MS/ICP-AES data reported a concentration in roots about 6 times higher for Ti than for Ag. μXRF analysis of root cross-sections revealed that the high Ti value was mainly due to the formation of an envelope of Ti around the root. Indeed, metal concentration studied with a laterally resolved technique proved that Ag is more internalized in the roots than Ti and thus potentially more translocated to edible organs. This technique also highlighted the different behavior of those two NPs; Ti was detected mainly as hot spots in the tissue and Ag had a more diffuse and homogeneous signal. This difference was substantiated by XAS analysis. Ag in the root was detected under two oxidation states from 100% Ag^0 to 100% Ag^I and in association with different ligands regardless of plant tissue. The predominant ligand identified by linear combination fitting was a thiol-containing molecule (glutathione), known to be implied in plant detoxification mechanisms. Inversely, Ti was detected unaltered with a TiO_2 anatase crystalline form. New information were also obtained on the effects of NP exposure on the ionome. Both NPs affected the ionome on a similar way, but on the case of Ti only for high concentrations. NPs reduced Cu, Zn and Mn concentrations in the parenchyma and vascular cylinder of the plants. All together and with an appropriate statistical analysis, those techniques permitted to deepen our knowledge about NP fate in plants and to obtain new and original data about their impact on plant ionome.

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Assessing the Paris city contribution to CeO2 and TiO2 nanoparticles concentrations in the waters of the Seine River by spICPMS and FEG-SEM imaging

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Engineered nanoparticles (ENPs) are of great interest because of their specific properties which make them useful especially in industrial area. For instance, cerium dioxide NPs (CeO_2NPs) can be found in the automobile domain and titanium dioxide NPs (TiO_2NPs) are used in cosmetics as an UV blocking filter. Nevertheless, these ENPs are expected to be found in the environment at modeled concentration of ppb or ppt levels (1). In order to detect these ENPs at relevant concentrations and among natural NPs, innovative analytical tools are required. A field investigation was done along the Seine River on May, 2015 from upstream to downstream Paris city. single particle Inductively Coupled Plasma-Mass Spectroscopy (spICPMS) (2) and Field Gun Emission - Scanning Electron Microscopy (FEG-SEM) are applied on the river water samples to detect and characterize CeO_2NPs and TiO_2NPs. Moreover, three tributaries (Orge, Marne and Oise rivers) are studied in order to see if there is a contribution of these rivers on the Seine River. Particle number concentration in the Seine River increases from upstream to downstream of Paris city for Ce and Ti. Indeed, 500,000 Ce-bearing part mL^{-1} and up to 1,350,000 Ti-bearing part mL^{-1} are measured at Marnay-sur-Seine, 96 km upstream Paris city. The highest particle concentration was found in the Marne River, a tributary of the Seine River, with 1,600,000 part mL^{-1} for Ce and 6,000,000 part mL^{-1} for Ti. This has an impact downstream the confluence between the Seine and the Marne rivers where the particle number concentration increases near Paris sampling point (1,400,000 part mL^{-1} for Ce and 4,700,000 part mL^{-1} for Ti). The next increase was observed near a water treatment plant (Bougival) with 1,200,000 Ce-bearing part mL^{-1} and 4,600,000 Ti-bearing part mL^{-1}, after Argenteuil sampling point (11 km downstream of Paris city). FEG-SEM imaging shows that particles containing Ce and Ti can be found in the suspended matter of the Seine River water. The size of detected particles varies from 200 nm to 5 μm. To further investigate the origins of NPs, elemental ratios of Ce/La and Ti/Al also need to be studied. 1. Gottschalk *et al*. 2013 ; 2. Mitrano *et al*. 2012

Wildlife ecotoxicology: from food chain exposure to population effects

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Effects of Pb exposure on sperm quality and reproductive output of red-legged partridge

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Lead (Pb) poisoning by the ingestion of shot pellets, bullet fragments or fishing weights is a frequent cause of death in wild birds, but also have a wide range of sublethal effects. Here we report on the effects that a sublethal Pb exposure during the prelaying period have on the breeding performance of red-legged partridges (*Alectoris rufa*). We studied separately the effects of exposing males or females on egg properties, laying performance, reproductive success, levels of dietary antioxidants and carotenoid-based coloration. We also studied the effects of male exposure on sperm quality and the relationship between sperm quality, carotenoid-based ornaments and antioxidant levels. We show that the prelaying Pb exposure induced the production of heavier and larger eggs, heavier chicks and reduced hatching success when females, but not males, were exposed. Fecundation rate and other laying performance parameters were unaffected. In males, Pb exposure decreased acrosomal integrity and sperm motility, and increased sperm vigour, but did not affect sperm viability, concentration or overall progress. Moreover, clutch size was increased in pairs in which the male had been exposed to Pb in comparison to unexposed pairs. Pb exposure increased levels of circulating antioxidants in males, whereas the percentage of eye-ring area pigmented by carotenoids decreased in exposed females. Overall, the sublethal Pb doses used here did not induce spermatozoon death or infertility in males, but rather caused an increase in reproductive investment. Pb exposed females also exhibited increased investment in reproduction, laying larger and heavier eggs and chicks, but had reduced carotenoid-based coloration and hatching rate. Several sperm parameters showed positive relationships with carotenoid-based coloration and levels of antioxidants that were influenced by Pb exposure, suggesting that redder males may be more capable to preserve sperm from oxidative stress.

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Mercury trends in seabird eggs from Pacific Canada, 1968-2012, and relationships with stable isotopes

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Seabird eggs are used to monitor environmental mercury because they are relatively easy to collect and integrate a signal from the entire food web. Trends in mercury from seabird eggs, however, may represent variation in diet rather than variation in mercury availability; due to biomagnification, seabirds switching to feed at a higher trophic level will usually have higher mercury levels. We measured mercury concentrations in eggs from six seabird species in Pacific Canada during the period 1968-2012. In contrast to expectation, storm-petrels feeding partially on invertebrates had the highest mercury burden while herons feeding on large fish had the lowest mercury burden. Rather than correlating with trophic level (δ^{15}N), mercury levels correlated with δ^{34}S ($R^2 = 0.86$). For cormorants, the only group showing a significant temporal mercury trend, both mercury and δ^{34}S decreased over time. Sulfate-rich environments (high δ^{34}S) are occupied by sulfate-reducing bacteria that produce methylmercury, and we hypothesize that variation in mercury within and among seabirds is associated with variation in methylmercury production by sulfate-reducing bacteria at the base of the food web. Variation in mercury levels in seabirds across space and time were associated with the origin of sulfur in the diet.

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Contamination of four ivory gull (Pagophila eburnea) colonies in Svalbard in link with their trophic behaviour

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The ivory gull is a high-Arctic species that spends the entire year closely associated with sea ice. This species is considered endangered in most parts of its breeding range. Ivory gulls must indeed cope with not only the reduction of sea ice cover triggered by climate change but also increasing contaminant loads due to changes in global contaminant pathways and the release of previously stored pollutants from melting snow and ice. In this context, the main objective of this study was to assess the trophic behaviour of this species in four colonies located

on Barentsøya, Svalbard, and the relationship between contaminant exposure and its diet. Contaminants, encompassing a number of organochlorines (OCs), brominated flame retardants (BFRs), and perfluorinated alkyl substances (PFASs), were determined in the blood (plasma and whole blood) of ivory gulls collected over several years. In parallel, carbon and nitrogen stable isotopes were determined in different tissues (feathers, plasma and red blood cells, or whole blood) to infer the trophic level ($\delta^{15}N$) and feeding habitat ($\delta^{13}C$) during both the breeding and the moulting periods. The most quantitatively abundant contaminants found in the ivory gull were *p,p'*-DDE (dichlorodiphenyldichloroethylene), ΣPCB (polychlorobiphenyl) and PFOS (perfluorooctane sulfonate). Ivory gulls demonstrated changes in the diet between the breeding and the moulting periods. The moulting period was characterized by enriched $\delta^{13}C$ and $\delta^{15}N$ signatures which suggest a more oceanic foraging location and a higher trophic position. This study also highlighted the same feeding habitats and strategies amongst ivory gull's breeding sites. The lack of differences in feather signatures between colonies could reflect either that birds from Barentsøya mainly come from the same migrating routes, or that they maintain the same diet independently of their wintering area. Several compounds including most of the PFASs, *trans*-nonachlor, *cis*-nonachlor, and BDE-28 demonstrated their biomagnification potential in the ivory gull food web. Overall, the levels of OCs, BFRs and PFASs did not suggest direct lethal exposure to these compounds but their potential synergetic or additive sublethal effects warrant continued monitoring.

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Development of Sublethal Thresholds and Toxicity Reference Values to Examine the Risk of Brodifacoum to Raptors

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On a global scale, brodifacoum continues to be one of the most widely used anticoagulant rodenticides for control of vertebrate pest species. Its toxicity and risk to non-target raptorial and scavenging birds is well-documented. While numerous exposure and toxicity studies have been conducted in owls (Stringiformes), its toxicity in other raptors (Falconiformes) in controlled exposure studies is less-well described. American kestrels (*Falco sparverius)* were provided daily access to two 25 g meatballs (Nebraska Bird of Prey diet) containing vehicle (control) or brodifacoum at nominal concentrations of 0.3, 1 or 3 ppm wet weight for 7 days (N=5 birds/group). These nominal brodifacoum concentrations were analytically verified (90.3-101.0% recovery), and are similar or less than carcass and liver concentrations (i.e., ~ 3 μg/g and ~18 μg/g, respectively) found in target rodents following eradication operations. Uneaten food scraps were collected daily, kestrels were observed twice each day, and weighed and examined at various intervals. After 7 days of exposure, a jugular blood sample was drawn into a syringe containing sodium citrate. Hematocrit was determined and plasma was frozen for clotting time assays. Birds were euthanized, necropsied and various tissues were fixed in phosphate-buffered formalin for histopathological evaluation. Neither food consumption nor body weight differed significantly among groups. Overt signs of intoxication (bruises on featherless tract, evidence of bleeding) and some microscopic hemorrhages were apparent at dietary concentrations of 1 and 3 ppm. In comparison to controls, hematocrit was reduced ($P< 0.05$) by ingestion of 1 and 3 ppm brodifacoum, with some birds being classified as anemic (hematocrit < 30). Prothrombin time and Russell's viper venom time were both prolonged ($P< 0.05$) in all groups receiving brodifacoum. Using data on daily brodifacoum consumption and classification of an exposed kestrel as being anemic, toxicity reference values (TRVs) were generated. The dietary-based TRV at which 50% of exposed kestrels exhibited anemia was estimated to be 246 μg brodifacoum consumed/kg kestrel body weight-day and 2.62 mg brodifacoum consumed/kg kestrel body weight-week. These TRVs are below environmental concentrations that might be encountered by free-ranging raptors consuming rodent following an eradication effort, and quantitatively document the hazard of brodifacoum to non-target birds of prey.

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Balance between ecological interactions and chemical exposure on food-chain dynamics

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In this study, we develop an ecotoxicological model based on a system of differential equations designed to predict the population dynamics of a terrestrial one-prey one-predator food chain exposed to metal contaminants. Biological models are small mammals, a raptor the barn owl, and cadmium (Cd), a persistent contaminant. We use our model to test different exposure scenarios of the predator according to prey trophic position (herbivorous, carnivorous or omnivorous). Contaminants, as the Cd, affect the survival and the reproduction abilities of exposed individual prey and predator, and therefore change the classical predator-prey dynamics. Analytical results show a general pattern where cyclic dynamics are observed at low concentration of Cd in soil, and then, the increase of Cd stabilizes populations dynamics. When the concentration of Cd increases, the model may have two stable steady points meaning that a subtle change in the initial conditions (initial prey and predator densities) implies totally differents steady states (predator-prey coexistence or extirpation of the predator). At higher contaminant concentration in soil, the biomagnification process extirpates the predator population what releases the predation pressure on preys and allows their increase. Finally, at high concentration, both prey and predator species collapse. The comparison of several prey species differentiated by their diet from herbivorous to carnivorous induces different patterns of predator exposure, and therefore change the dynamics of the entire food-chain. The results obtained from this food chain model illustrates the importance of cascades effects, that is indirect effect modulated by trophic relationships. For instance, the model reveals the occurrence and position of tipping point (bifurcation) where a subtle change in conditions causes a sudden change in the ecological system. The exploration of the tipping point behaviors with a mathematical bifurcation analysis allows to measure the impact of a change in prey resources (bottom-up cascade effect) or in the apex predator abundance (top-down cascade effect). As a consequence, this simple food chain model can help to identify populations that are critical for transfering adverse effects of contaminants across trophic levels. The direct next step of this work is the incorporation of multi preys though the use of multi-species functional responses to model the prey-selection behavior of the top predator.

Mechanistic effect modelling for risk assessment: state of the art, applications, use in a regulatory context and future directions (II)

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BEEHAVE model evaluation according to Good Modelling Practice

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Rapid scientific progress has been made in recent years in ecological modelling. Workshops such as Lemtox and MEMORISK have explored the use of ecological models in pesticide risk assessment. Industry has submitted models to address specific risk assessment questions. However, the lack of guidance on the evaluation of models has been a major hurdle for using them in a regulatory context. In order to facilitate the evaluation (and the development) of regulatory ecological models EFSA issued an opinion on good modelling practice[1] or GMP. The GMP approach was first applied in the EFSA PPR panel statement [2] to evaluate BEEHAVE [3], a comprehensive individual based model simulating honeybee colonies that was published in 2014 (http://beehave-model.net/). The BEEHAVE model fulfilled most of the criteria outlined in the GMP opinion [1]. It was concluded that the model is not yet usable in a regulatory context or to address the risk from multiple stressors at the landscape level. Recommendations for further developing the model in order to address these questions are, for example, the development of a pesticide module, adding additional biological agents such as infectious agents, pests and predators and interactions between those agents and the landscape (including critical beekeeping practices, climate, weather and landscape characteristics).The supporting data and default parameter values should be further evaluated and justified. The PPR Panel recommends adopting the model as the basis for modelling the impact on honeybee colonies of pesticides and other stressors. Further development might require a different programming language, for example a standard object-oriented language.

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The honeybee model BEEHAVE: current status and future developments

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The honeybee model BEEHAVE was developed to explore the influence of various stressors on colony health and survival. The main stressors represented are limitations in forage, diseases transmitted by varroa mites, and pesticide-induced mortality of in-hive bees or foragers. An expert working group at EFSA evaluated in great detail the suitability of BEEHAVE for regulatory risk assessment. Because of this potential of and interest in BEEHAVE, we here summarize the state of the art regarding BEEHAVE and outline possible future developments. BEEHAVE was used to (1) explore the effects of forage gaps and (2) pesticide-induced mortality on colony survival. Forage gaps of two weeks in June or July are most detrimental. Increased mortality of adults affected colony survival much more than pesticide effects on the brood or egg-laying. EFSA considered the representation of honeybee colony dynamics and foraging suitable, but would require a specific pesticide module and an easier representation of different landscape before BEEHAVE can be used in regulatory risk assessment. We conclude that BEEHAVE is a milestone towards using mechanistic effect models for risk assessment for honeybees, but further model tests, refinements, and possibly a re-implementation are needed.

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What can we learn from robustness and sensitivity analysis of an individual-based Collembola model for risk assessment?
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With respect to environmental risk assessment it is crucial to know where and when to protect an organism but still little is known on the vertical dispersal and seasonal fluctuations of collembolan communities in agricultural landscapes. To overcome this lack of knowledge, we developed an individual-based model of the soil-dwelling collembolan *Folsomia candida*. The model FOLCAS (Folsomia candida simulation) is a vertical distribution model in an agricultural soil column, which can be applied to demonstrate the effect of variations in environmental parameters on the population and its dispersal. In addition, the model features the option to evaluate the effect of a pesticide application. In this study we will present the results of the first robustness analysis and sensitivity analysis of the model FOLCAS. We will use the findings to improve the model, but also to learn about their possible impact on the current risk assessment of non-target arthropods. It is assumed that the model reflects the major environmental processes. Thus, the sensitivity analysis allows us to review the hypotheses of influencing factors on the collembolan movement and hence its possible exposition to a plant protection product within the field. The method of sensitivity analysis not only enhances the understanding of the model, but it can also highlight the important processes and parameters, which are crucial to establish a more realistic environmental risk assessment for soil-dwelling collembolans. Once identified, these sensitive processes and parameters need further attention in order to reduce the uncertainty of the risk assessment. Parameters or processes identified as insensitive for the system can be assumed less important, where even high insecurities will not lead to a high uncertainty of the risk assessment.

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A model-based method to analyze ecotoxicology experiments in mesocosm
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The mesocosms (experimental ecosystems) improve the ecological relevance of the studies on the effects of xenobiotics on aquatic species. However, in mesocosm studies, the number of replicates is limited by practical and financial constraints. In addition, high levels of biological organization are characterized by a high variability of descriptive variables. This variability and the poor number of replicates have been recognized as a major drawback for detecting significant effects of chemicals in mesocosm studies. These characteristics induce a large uncertainty on the probabilistic distributions of the endpoints (location parameter, variability and shape) in mesocosm experiments. To reduce this uncertainty, a solution would be to optimize the knowledge used to define the expected probabilistic distribution of the endpoints for a given experiment in control conditions. A way to combine all the information available is to develop, calibrate and validate a model of for the population studied in mesocosm. This model will be fed by the characteristics of a given experiment (initial points, environmental *scenario*), the characteristics of the species that was chosen (ethology, ecology, population dynamics) and the characteristics of the experimental system (for example species composition of the mesocosm). We evaluated here this methodology on experiments conducted in lotic mesocosm and focused on the three-spined stickleback (*Gasterosteus aculeatus*)population dynamics. An individual based-model (IBM) was developed from a bioenergetic model following the Dynamic Energy Budget theory in order to represent the life-cycle of sticklebacks (DEB model). Sensitivity analyses were performed to explore the DEB-IBM, and the parameters of the model were adjusted using two dataset with two different environmental *scenarii*. The predictability of the model was then tested using two other datasets. Finally, we observed on a case-study that mesocosm data analysis was improved by using the model-based methodology proposed. To conclude, designing an individual-based model is very promising for improving mesocosm data analysis.

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Modelling the impact of herbicides on phytoplankton for relevant ecological scenarios of varying complexity
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Mechanistic effect models (MEMs) are useful tools for ecological risk assessment of chemicals. The most important recommendations of the SETAC MODELINK workshop are that the models should be as realistic as possible for a specific risk assessment question, and the level of conservatism required for a specific risk assessment should be reached by designing appropriately conservative environmental and exposure scenarios. As for aquatic invertebrates, phytoplankton algae have to be protected at the population level by considering their abundance/biomass in edge-of-field surface waters [EFSA AGD]. Especially for the ecological recovery option (ERO), all relevant processes that determine population viability and the propagation of effects on the community-, ecosystem- and landscape-level must be considered. As defined in the EFSA AGD the reference tier for algae are micro-/mesocosms including competition, predation and natural stressors. According to these requirements, aquatic algae should be modelled as populations within an ecosystem modelling framework. The main question to be addressed is the level of complexity which is needed for a realistic and accurate description of population dynamics. In this presentation, we will introduce an ecosystem model framework for quantifying responses to stress at different levels of ecological complexity for freshwater algae in standing waters. We use a complex biogeochemical lake model (StoLaM) to predict population dynamics of phytoplankton species under realistic field conditions taking into consideration growth rate inhibition as the toxic mode of action. StoLaM's modular structure enables us to simulate a wide range of ecological scenarios from laboratory conditions (one algal species, constant temperature and light conditions) to high ecosystem complexity with several competitors, trophic levels and dynamic physico-chemical and weather conditions. Our aim is to develop ecological scenarios of increasing ecosystem complexity using StoLaM, and to evaluate these scenarios under different exposure and climatic conditions with respect to the sensitivity of the phytoplankton.

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Simulation of Exposure, Bioaccumulation, Toxicity and Estimated Productivity Losses from the Deepwater Horizon Oil Release in the Mississippi-Alabama Nearshore Marine Environment
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The Deepwater Horizon (DWH) incident provided an opportunity to develop the mechanistic model, AQUATOX 3.1 Nearshore Marine Environment (NME), to estimate impacts from this event. Oil exposure followed the generally accepted conceptual framework that the oil rapidly coursed across the surface of the water column towards the shoreline with mixing by winds, waves and currents. This relatively short-lived water column event was followed by a subsequent pulse of contaminants, primarily various PAHs, which became re-entrained in the detrital and sediment pools within the nearshore coastal environment. The PAHs were then diluted by advection and various degradation processes and were taken up by the food chain, as was borne out by the available data collected during and after the incident. We present our approach, using the AQUATOX 3.1 NME modeling framework, to analyze this oiling event with water-column exposure followed by a longer-lived sediment exposure to the food chain. Exposure to nearshore coastal habitats uses maximum-likelihood estimates of various scenarios of oil concentrations. The octanol-water equations for partitioning of PAHs in the water column (dissolved and various combined forms) within various PAH "bins" are presented. Using AQUATOX 3.1 NME, we then predict subsequent uptake and bioaccumulation of the PAH bins within various biotic groups and predict resulting toxicity. Observations of water, sediment, and biota available through the natural damage assessment and other published sources are used to verify the model predictions. From this exposure and the subsequent toxicity we have estimated overall productivity losses at several trophic levels across various habitats within the nearshore Mississippi-Alabama coast.

Quantitative in vitro to in vivo extrapolation (QIVIVE): Advances in tools to quantify exposure (dose)-response relationships and use in risk assessment

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Building improved in-vitro exposure assessment capability: Towards the development and implementation of enhanced QIVIVE tools
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Risk assessment is generally divided into human health risk assessment (HRA) and environmental risk assessment (ERA). For instance, scientific research, policy discussions, and regulatory instruments for HRA and ERA are addressed by individuals with different sets of expertise and knowledge, oftentimes at different institutes, research organizations, and government agencies. Integration of HRA and ERA, however, could provide substantial benefits, particularly by providing a more efficient framework on which to address emerging problems and questions that have the potential to impact both the environment and human society. Society is facing a variety of challenges; growing concerns about the effects of multiple

stressors (both chemical and non-chemical); risks associated with exposure to complex mixtures; and demands to quantify local site-specific risks. Integration of HRA and ERA could thus lead to more scientifically sound assessments by combining cross-discipline expertise and data, lowering costs and reducing the time needed to improve quantification of risks. A key challenge, however, in advancing our understanding between toxicological effects and exposure concentrations is the need for improved tools to measure and control the freely dissolved concentration of a chemical, particularly in an *in vitro* test system. Current practice typically relates results obtained from an *in vitro* test system to a nominal concentration, which acts as a surrogate in establishing exposure-response relationships. However, depending on the physicochemical properties of a chemical, substantial variability between the nominal and the freely dissolved concentration might exist. Characterizing uncertainty related to the difference between the nominal and freely dissolved concentration is necessary to advance our understanding of integrating hazard and exposure information. This also represents a challenge of quantitative *in vitro*-to-*in vivo* extrapolation (QIVIVE) tools, which are necessary to enhance the development of mechanistic physiologically based pharmacokinetic (PBPK) models. This presentation will explore research needs related to quantification of the key processes influencing the exposure-response relationship in a variety of *in vitro* test systems. The objective is to help prioritize research needs that will address the limitations associated with improved quantification of in vitro to in vivo extrapolation.

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Examining underlying assumptions when translating in vitro bioassay results to in vivo conditions

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There is a paucity of measured toxicity data compared to the large number of chemicals and endpoints required for chemical hazard assessment. Emerging alternative test methods are being developed and evaluated to reduce and eliminate unnecessary animal testing. Cell-free and cell-based in vitro systems are being developed and applied for hazard and risk assessment. For human health assessment nominal concentrations from these in vitro assays are commonly assumed to be equivalent to steady-state blood concentrations in vivo. These steady-state blood concentrations are then used to calculate oral equivalent doses (OEDs) that can then be compared against exposure intake rates for risk-based prioritization. Here we use in vitro mass balance models and equilibrium partitioning theory to examine differences in the dissolved chemical concentration corresponding to the assumed nominal (administered, unmeasured) chemical concentration from an in vitro assay for a range of chemical partitioning properties and in vitro assay conditions. We compare the in vitro concentrations to in vivo blood concentrations and illustrate how the data can be interpreted differently. Finally, we apply the chemical activity approach in the data analysis as a means to clarify data interpretation and translation across systems (i.e., in vitro to in vivo). Implications for hazard and risk-based ranking are discussed.

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Bioavailability of organic micropollutants in cell-based bioassays

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In vitro bioassays are generally complex heterogeneous systems, and differences in chemical distribution are recognized as important issues concerning *in vitro* bioavailability. The freely dissolved concentration is generally considered as responsible for biouptake and can be reduced by sorption to medium compartments, biomaterial and plate material as well as evaporation and degradation. Consequently, the nominal concentration is often not an adequate metric to describe exposure and characterize toxicity. Our approach is to apply and experimentally validate a full mass balance model to different cell-based bioassays covering various toxicity pathways and a selection of non-volatile priority chemicals. We will then use the model to link observed toxicity directly to modelled bioavailability. The first stage of the model is based on equilibrium partition constants, with kinetic processes, such as degradation and diffusion into plate material excluded. We collected partition constants from the literature or used Quantitative Structure-Activity Relationships and other models for prediction. Bovine serum albumin and liposomes (membrane lipid bilayer vesicles) served as surrogates for proteins and lipids in the test medium and cells. The protein and lipid concentrations in the medium and cells were measured. Chemicals with low hydrophobicity were predicted to be predominantly freely dissolved and thus bioavailable. With increasing hydrophobicity, the fraction of chemicals bound to proteins and lipids increased strongly, with fractions of freely dissolved chemicals < 0.1 % for chemicals with a log K_{ow} > 6. These results demonstrate the deficiency of using nominal concentrations to characterize toxicity, in particular for hydrophobic chemicals, whereas correcting effect concentrations by modelled freely dissolved and cellular concentrations can lead to higher significance and comparability of toxicity data. We aim to improve the accuracy of the model in the near future. For this purpose, partition constants for chemicals with diverse physicochemical properties to medium compartments and cells will be measured. We will optimize and generalize the bioassays to which the model will be applied and produce empirical data on medium composition and structure of the respective cell lines. Furthermore, time-dependent processes like diffusion in plate materials, degradation, cell-uptake and growth dilution will be considered.

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Getting biotransformation kinetic parameters as a bonus out of bioaccumulation experiments

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Biotransformation is one of the important processes that can influence the fate of contaminants in living organisms. To properly understand the contribution of biotransformation, the *in vivo* metabolic rate constant, k_M, needs to be determined. Despite its importance, k_M has remained one of the least understood (in terms of modeling) and one of the most hard-to-get parameters. Two major approaches are currently and commonly used to determine k_M: by *difference* and by *experiment*. The *difference* approach derives k_M by subtracting the contributions from growth (k_G), egestion (k_E), and out-going transport from the biota (i.e., k_2) from the total depuration constant (k_T). While this approach has been successfully applied, leading to the development of k_M database (e.g., Arnot et al. 2008 ET&C 27:341-351), it has the occasional problem of producing negative k_M's. The *experiment* approach, while preferred in principle over the *difference* method, suffers from a lack of mathematical tools to properly delineate k_M from the observable quantities. Furthermore, it is often complexed with experimental constraints intrinsic to standard bioaccumulation procedures or practices. We proposed a generic protocol, developed a set of mathematical models, and devised a scenario map to address the need for determining k_M experimentally. Equations were constructed from first principle following the established first-order kinetic framework for different exposure and accumulation scenarios. The developed approach allows k_M of the parent compound (PC) to be determined from the kinetic measurements of its metabolites (MBs). The protocol and the scenario map were applied to and successfully validated using data from studies where PC and MB kinetic data were reported. This study ended with recommendations on how bioaccumulation of contaminants in living organisms may be experimentally investigated to reduce resource, effort, and data-reduction complexity while getting accurate toxicokinetic properties of the contaminant. Our message is that k_M's can be easily determined as a "bonus" if bioaccumulation experiments and data reduction are done right.

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Mapping risk assessment challenges for HPC ingredients: a chemical space analysis

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The inclusion of chemical ingredients in Home and Personal Care (HPC) products is based onthe functionality that their individual physicochemical properties bring towards enhancing the overall performance of the product. Given the broad range of functions that HPC products provide (cleansing, moisturizing, conditioning, etc), chemical ingredients used in these products can therefore capture a broad range of chemical classes, for instance from being extremely hydrophilic to extremely hydrophobic, neutral organics, inorganics, ionisable, and permanently charged salts. As it happens, not only do the physicochemical properties of various chemical ingredients influence the functionality of a HPC product, they also influence the behaviour and fate in test systems (*in vitro*, *in vivo*, and *in silico*) and in the environment. It is thus critical that the physicochemical properties be considered in both experimental design and selection of appropriate in silico tools. Using an example from industry, we examine the chemical space of a selection of HPC ingredients (>7000) and discuss implications towards assessing behaviour *in vitro*. We base our analysis on batch estimates of chemical properties (using SMILES strings), and discuss the validity of such estimates for the chemicals in question. We filter the chemicals using a set of basic criteria to identify groups of chemicals for which standard risk assessment procedures are not applicable. This analysis provides a comprehensive overview of the specific modelling and laboratory research challenges that risk assessors face in dealing with HPC ingredients.

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Regulatory Integration of In Vivo and In Vitro Toxicity Information: A Perspective on the Extent of the Challenge

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Formulating regulatory exposure levels for environmental media on a chemical-by-chemical basis has been successful. However, changes in emphasis - reducing test animal usage, focusing on much larger numbers of chemicals, and addressing multiple media and species - require a more efficient regulatory paradigm and more testing data. Alternative approaches being considered involve generating large amounts of *in vitro* toxicity data, then linking changes in biochemical processes through levels of biological organization (LBO) to whole organism adverse apical effects and beyond – populations, communities, and ecosystems. As toxicological understanding improve proponents aspire to move from *in vivo* toxicity testing prioritization to supplantation. New paradigm challenges, and recommendations to address them, are reviewed in four areas. Firstly, dose-response difficulties. Bioavailability is an important confounding influence, affecting the dose surrogate chain from external exposure through whole organism then organism subcompartments to the subcellular site(s) of toxic action. Additionally, solvents used in most *in vitro* testing protocols have an unquantified bioavailability influence that can act as a toxicity modifying influence. Secondly, there many toxic action identification/classification schemes. However, there is no holistic, phylogenically-relevant (human/mammalian and environmental; i.e., plants and bacteria through fish to birds), broadly applicable, widely accepted scheme for identification, classification, and relative potency estimation for either modes/mechanisms of toxic action or adverse outcome pathways (AOP). Thirdly, in the LBO concept new properties that emerge from translevel integration of lower level properties are not necessarily predicable. Thus, it is difficult to establish single direct deterministic causal links through multiple LBO levels. Effects observable at upper LBO levels may be induced by multiple unrelated initiating events occurring at lower LBO levels. Also, *in vivo* toxicology is a “middle-out” approach with an inordinate focus on tests whose outcomes are for narrowly defined statistical populations poorly linked to higher LBO. Fourthly, is the largely neglected issue of primary validity and task-specific relevance for both *in vivo* and in vitro toxicity testing data. Use of accepted testing methods is not a guarantee of validity or relevance and weight-of-evidence (WOE) schemes rarely address either adequately.

Epigenetic and evolutionary effects of pollutants: new challenges for long-term ERA

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Integration of Epigenomics in Systems Toxicology

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Increasing evidence demonstrates that environmental insults occurring during early development may have life-long effects, indicating that epigenetic mechanisms could be involved. However, epigenetic approaches in ecotoxicology are still lacking. Therefore, we engaged in a study to evaluate the effects of Endocrine Disruption Chemicals (EDCs) on zebrafish development by combining transcriptomics and epigenomics. Applying Next Generation Sequencing (NGS) technologies, we aimed to: 1) recognize target genes regulated by EDC exposures at the transcriptomic and epigenomic level; 2) develop new tools to characterize toxicity pathways and identify epigenetic biomarkers; 3) integrate transcriptomic and epigenetic platforms to identify whole genome molecular footprints characteristic of EDCs exposures. Due to its similarities in epigenetic regulatory machinery to mammals, the zebrafish is a recognized model for the analysis of epigenetic mechanisms in developmental biology and in environmental epigenetics. Our long-term target is the fusion of multiple omic techniques to identify models to predict phenotypic traits and outcomes. For example, the simultaneous determination of expression patterns by RNA-sequencing (RNA-seq) and differentially methylated genes by DNA Immunoprecipitation sequencing (MeDIP-Seq) allows the study of epigenetic effects at different levels and the identification of new pathways that might not be clearly identified by the separate analysis of the two data sets. Results presented here will provide a unique possibility to increase the understanding of the new field of ecotoxicoepigenomics and will exert a positive influence in the study of the effects of emerging contaminants in the genome as a whole. This study will increase the knowledge of regulatory mechanisms and modes of action of EDCs at a global scale, including epigenetic effects. Downstream analysis such as DNA motif discovery or the prediction of protein function could be proposed in the future to uncover potential modes of action. We anticipate that studies on ecotoxicoepigenetics will assist in early detection and risk assessment of environmental emerging contaminants in the near future.

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Differential DNA methylation following mono(2-ethylhexyl)phthalate and 5-azacytidine exposure in zebrafish.

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Recent rodent studies demonstrating environmentally induced transgenerational epigenetic effects linked to increased disease risk have drawn much attention. Some of those studies observed changes in the DNA methylome indicating that effects can be inherited via DNA methylation. However, most of these studies use high concentrations and some results cannot be replicated in follow up studies. Clearly, more research is needed to understand the basic mechanisms encompassing transgenerational epigenetics. Here, we used a transgenerational set up using zebrafish as a vertebrate model organism to investigate the acute, latent and transgenerational effects on gene expression and DNA methylation of early life exposure to the putative obesogenic phthalate metabolite mono(2-ethylhexyl)phthalate (MEHP) and the DNA methylation inhibitor 5-azacytidine (5AC). We exposed zebrafish from 0 to 6 days post fertilization (dpf) at concentrations, which did not cause embryotoxicity (30 and 10 μM for MEHP and 5AC, respectively). We analyzed the expression of 41 obesity related genes, performed global (hydroxy)methylation analysis and genome wide DNA methylation analysis (reduced representation bisulfite sequencing) in F0 6 dpf larvae. Analysis of gene expression and global methylation in F0 larvae (15 dpf) and adults (brain, gonads) as well as F1 and F2 embryos is currently underway. In addition, we performed bisulfite amplicon sequencing on 10 differentially methylated regions (DMRs) discovered by the genome wide methylation analysis. Our results show a high number of genes (18 out of 41) were differentially expressed at 6 dpf, but only two of those genes were differentially expressed at 15 dpf following MEHP exposure. Subsequent analysis is ongoing. Genome wide DNA methylation analysis revealed 148 and 311 DMRs for MEHP and 5AC, respectively. Ingenuity pathway analysis revealed enrichment in cancer development, gastrointestinal diseases and nervous system development with MEHP, as well as a significant enrichment of genes related to obesity. For 5AC, general stress responses and effects on embryonic development were enriched. Bisulfite amplicon sequencing is ongoing and will reveal whether selected regions are transgenerationally inherited. Our results reveal considerable effects on DNA methylation following exposures during early life in zebrafish to MEHP and 5AC at nontoxic concentrations. This study will provide new knowledge about transgenerational epigenetics in zebrafish.

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Genetic and epigenetic modifications following parental exposure of the Pacific oyster, Crassostrea gigas, to the herbicide diuron: The GIMEPEC project

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Oyster production basins are located in coastal waters receiving pollutants from exoreic catchment area. Among them, pesticides such as herbicides are of concern as they are frequently detected and sometimes at high concentrations. As a consequence, there is a need to study their impact on oyster. As a matter of fact, recurrent oyster mortality outbreaks are observed worldwide, legitimating the suspicion of a possible impact of pollutants on its ability to face environmental stressors. That is the main goal of the GIMEPEC « Genotoxicity, IMmunotoxicity and rEprotoxicity of PEsticides in *Crassostrea gigas* » project that puts forward genetic alterations induced by herbicides as a basis for a potential decrease in oyster physiological performances. To investigate the parental effects of herbicides, genitors involved in gametogenesis were exposed to environmentally realistic pulses of diuron. At spawning, semen from each control and assay groups were recovered in order to produce the next generation. Among results, we first demonstrated the genotoxicity of diuron by the comet assay in the hemocytes of oyster genitors. Moreover, the oxidised DNA base 8-oxodGuo was also detected at higher levels in the gonads of genitors exposed to the herbicide. Immunohistochemistry analysis revealed the presence of 8-oxodGuo in all stages of germ cell line, explaining the detection of DNA damage in reproductive cells as well: in spermatozoa of exposed genitors a significantly higher level of DNA strand breaks was measured. The vertical transmission of diuron-induced DNA lesions was confirmed by flow cytometry analysis of offspring DNA. Both a clastogenic (chromosome breakage) and aneugenic (change in chromosome number) effect was detected following parental exposure to the herbicide. By using Fluorescent In Situ Hybridization, it was even possible to identify some of the damaged part of oyster genome. In parallel to this genetic modifications, parental exposure to diuron affected DNA methylation in the offspring. A BS-seq analysis allowed the identification of 235 differentially methylated regions corresponding to 169 genes. By comparing these results with those from RNA-seq, no clear relationship was however showed between methylation and gene expression. These modifications of the DNA were concommitally observed

with negative impacts on spat physiology in terms of reduced growth (reversible) and developmental abnormalities.

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Field evidence of reproduction impairment through sperm DNA damage in the fish nase (Chondrostoma nasus) in anthropized hydrosystems
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A clear trend for a worldwide decline of fish populations has been documented over the last decades in freshwater ecosystems. Among numerous potential causes, pollution pressure due to industrial, agricultural and urban activities is considered as one of the main factors responsible for this decline. Contaminants can affect fish reproduction through different mechanisms leading to endocrine disruption and/or to the impairment of embryonic development, hatching and larvae growth. Previous studies have demonstrated a linkbetween the integrity of sperm DNA and the quality of offspring development in fish and invertebrates. Present work aims to explore this relationship in the field as a possible cause of the decline through reproduction impairment of a feral fish population. Mature nase (*Chondrostoma nasus*) were caught during the breeding season in three locations (A, B and C) of the Rhône River basin and gametes were collected by stripping. Sampling locations were chosen according to the following gradient of contamination due to human activities on the watershed: A≤B< C. Samples of a pool of collected ovocytes were fertilized with the sperm of individual males and then incubated individually back to the lab to study embryo-larval development as well as sperm samples to assess DNA integrity. Genetic analysis clearly showed the absence of a difference in genetic structure between the three studied fish populations from the Rhône basin. Sperm DNA integrity was significantly lower in males from station C compared to other ones when sperm biochemical characteristics and fertilization rate remained globally unchanged whatever the station. Mortality and abnormality rates measured at both hatching and at the end of yolk sac resorption stages followed the same trend than the sperm DNA damage, demonstrating an impact of river water quality on nase fitness through a loss of sperm DNA integrity. Since the level of both abnormalities and mortality measured in offspring of fish caught in the most contaminated area reached high values up to 15 % and 80 % respectively, the hypothesis of an observed nase decline in Rhône River stemming through selection forces can be put forward.Possible modifications in habitat and food resources threatened by river regulation and climate change, and immunosuppression triggered by high level of chemical pollution should be kept into account for a valuable assessment of human activities consequences on aquatic ecosystem functioning.

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Mechanistic links between molecular alterations and transgenerational effects of depleted uranium and radionuclides in Daphnia magna
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Understanding how toxic contaminants affect species at sub-cellular, histological, physiological, organism, population levels of biological organization is a major research goal in both ecotoxicology and radioecology. Mechanistic links among the observed perturbations are necessary to predict consequences for survival, growth and reproduction which are critical for population dynamics. Time scales at which such links are studied are rarely relevant for natural biota. With a small size and short life cycle, the cladoceran crustacean *Daphnia magna* is suitable for studying contaminants effects over several generations. Multigenerational toxicity studies are much more relevant to natural biota for which exposure can involve many successive generations. Multigenerational studies of toxic effects were conducted under controlled conditions in *D. magna* exposed to depleted uranium (U), americium-241 (Am-241) and cesium-137 (Cs-137), representing respectively a dominantly chemotoxic metal, an alpha internal contamination and a gamma external radiation. Results showed in all cases that toxic effects on physiology and life history (survival, body size, fecundity) increased in severity across generations. These observations demonstrated that measured effects in one generation might not be representative of toxicity in the following offspring generations, and ultimately of the population response. Reduction in somatic growth and reproduction induced by depleted U were analyzed using a DEBtox approach (dynamic energy budget applied to toxicology). Modelling results suggested that depleted U primarily affects assimilation. This metabolic mode of action was confirmed by measurements of assimilation reduction and observations of histological alteration of the digestive epithelium. However the mechanisms involved in the transgenerational increase in sensitivity remained unknown. Recent studies investigating DNA damage in daphnids exposed to depleted U and Cs-137, demonstrated that molecular alterations were accumulated in females over the course of exposure and transmitted to their progeny. Such alterations were interpreted as the underlying mechanism causing the increase in effect severity over generations and will be investigated, as a future perspective, during exposure to Am-241. DEBtox models considering the accumulation and transmission of genetic damage were used to analyze toxic effects of depleted U, Cs-137 and Am-241 in daphnids exposed over successive generations.

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Evolution of Lymnaea stagnalis inbreeding depression under pesticide chronic exposure
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Ecological and genetic factors may modulate the toxicity of chemicals to natural populations. In particular, inbred populations may be more sensitive to pollutants than their outbred conspecific counterparts, due to high homozygosity, loss of genetic diversity, or increased genetic load. On the other hand, strong inbreeding, such as expected in highly selfing hermaphrodites, may have a positive effect on population response to chronic (multigeneration) exposure to toxicants. First, selfing may facilitate the response to the selection exerted by a toxicant, through a transmission of coadapted gene complexes more efficient than under random mating. This is due to the fact that selfing reduces effective recombination. Furthermore, evolutionary theory predicts that spontaneous deleterious mutations can be preferentially purged under selfing, whereas they are maintained for a longer time within outcrossing populations, as masked in heterozygotes. Thus, high and longlasting inbreeding may have positive effects on fitness, and possibly on the ability to face chronic environmental stress. We tested these hypotheses in the freswhwater gastropod *Lymnaea stagnalis*, using a three-generation exposure to the pro-oxidant herbicide diquat. A total of 16 lineages derived from a highly diverse genetic pool were either exposed to diquat at early stages or maintained under control conditions. Chemical exposure was crossed with the mating system, i.e., in each treatment (control, diquat), four lineages were forced to self-fertilize and four other lineages were allowed to outcross. After three generations, inbreeding depression, the fitness decrease of selfed progeny relative to the outcrossed one, was compared across treatments. Inbreeding depression increased under diquat exposure, reflecting an aggravation of the toxic effects under inbreeding. In other words, selfing did not improve the response to selection by diquat. However, selfed lineages also showed an increase in trait variance, especially under diquat exposure. Some inbred lines performed better than some outcrossed lines, suggesting that the response to selection might depend on lineage genetic composition. In terms of toxicity testing, the use of strains maintained for generations in the laboratory may be questionable, due to high inbreeding and depleted genetic variability. To circumvent this drawback and improve ecological risk assessment, we recommend the use of at least two distinct original populations.

Endocrine Disruptors: Exposure, Hazard & Risk Assessment (II)

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Thyroid disruption in zebrafish (Danio rerio) larvae: different molecular initiating events leading to impaired eye development and visual functions
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The vertebrate thyroid hormone system is important for multiple developmental processes, including eye development. Thus, its environmentally induced disruption may impact important fitness-related parameters like visual capacities and behaviour. The present study investigated the relation between initiating molecular events of thyroid disruption and morphological and physiological changes of eye development of zebrafish (*Danio rerio*). Two test compounds representing different molecular modes of thyroid disruption were used: propylthiouracil(PTU) as enzyme-inhibitor within thyroid hormone synthesis, and tetrabromobisphenol A (TBBPA) as ligand of thyroid hormone receptors. Both test chemicals significantly altered transcript levels of thyroid-related genes in a compound-specific way. Despite differing molecular response patterns, both treatments resulted in similar pathological alterations of the eyes such as reduced size and pigmentation, which were concentration-dependent. The morphological changes translated into impairment of swimming activity and visual performance of the larvae: the optokinetic response was significantly and concentration-dependently decreased in both treatments, together with a significant increase of light preference of PTU-treated larvae. This study provides first evidence that thyroid disrupting compounds do not only impair morphological but also functional eye development in fish early life stages, and that the phenotypic outcomes appear to be similar for different modes of molecular action of the thyroid disruptors.

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Obesogenic effects in Daphnia magna. An emerging endocrine disrupting effect
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Assessment of endocrine disrupters has been largely focused on vertebrates and on disruption of the hypothalamic-pituitary--gonadal/thyroidal axes of laboratory animals. There are, however, many compounds able to disrupt other nuclear receptor signaling pathways. An important group of endocrine disrupters are obesogens that disrupt the retinoic X receptor and PPAR and cause obesity in vertebrates. There is, however, little evidence of obesogenic effects in non vertebrates.. A recent study showed that tributyltin (TBT) activated the ecdysteroid, juvenile hormone and retinoic X receptor signaling pathways, and disrupted the dynamics of neutral lipids in the crustacean *Daphnia magna* impairing the transfer of triacylglycerols to eggs and hence promoting their accumulation into lipid droplets in post-spawning females. Tributyltin disruptive effects translated into a lower fitness for offspring and adults. The present study aims to addresses the disruptive effects of existing compounds alone and in mixtures on the lipid droplet dynamics in post-spawning females and their health effects.*D. magna* individualswere exposed 12 chemicals that included vertebrate obesogens (TBT, triphenyltin, bisphenol A (BPA), 4-nonylphenol (NP), di-2-ethylhexyl phthalate), other contaminants known to affect arthropods (pyriproxyfen (PP), fenarimol (FEN), methoprene (MET), emamectin benzoate (EM and fluoxetine (FX)), as well as the natural hormones methyl farnesoate (MF), 20-Hydroxyecdysone (20E). Reproductive effects were assessed by Life History analysis methods. Quantitative changes in storage lipids were studied using Nile red staining and ultra-performance liquid chromatography coupled to a time-of-flight mass spectrometry. Ten compounds disrupted storage lipid in a concentration related manner enhancing (TBT, MF, PP, BPA, 20E) or decreasing (NP, FEN, EM, MET, FX) their accumulation into lipid droplets in post-spawning females. Joint binary mixture effects indicated that the studied compounds acted on storage lipids additively and non-additively disruption the signaling pathways of ecdysone, methyl farnesoate and retinoic X receptors. In eight compounds disruptive effects translated into detrimental effects in growth and or reproduction.
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Occurrence of alkylphenols, volatiles, and metals in tap water in households with epoxy coated water pipes

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Organic chemicals in tap water originate from several sources. Organic pipe materials are a potential source of contaminants. Compared to communal distribution system small water pipes in households have potentially greater impact on water due to higher surface-to-volume ratio [1]. Organic pipe materials include pipe inner surface lining materials such as epoxy resins. Epoxy lining has been used as a cost-effective alternative to renovate old water pipes. Most common component in epoxy resins is bisphenol A (BPA). Leaching of chemicals such as the endocrine disruptor BPA has been shown to occur from epoxy linings in pipes [2]. In addition, epoxy liners of food and beverage cans has been shown to leach BPA to the contents [3]. Consequently, long-term durability and safety of drinking water pipe epoxy linings has been a topic of discussion. In this study drinking water from apartment houses with different age epoxy linings were studied. Samples were collected in summer 2015 from 6 houses with epoxy lined drinking water pipes and a drinking water treatment plant (DWTP) in Helsinki, Finland. Tap samples were collected after about 8 hours of discontinued water usage and after 2 min flowing, both on cold and hot lines. For comparison further sampling were done in December 2015 at 5 other sites with different age and material pipes. Samples were analyzed for alkylphenols BPA, bisphenol F (BPF), 4-t-octylphenol (OP), and 4-n-nonylphenol (NP) with HPLC-MS [4], and for volatile organic compounds, and metals (IPC-MS). Total (anti)estrogenic and (anti)androgenic potency and BPA-like activity will also be tested with yeast-cell-based assays [5, 6]. Bisphenol A was frequently detected in water of houses with epoxy lined pipes. DWTP samples had no detectable BPA. Incoming water and cold flowed water had less than 16 ng/L of BPA, while pipe-incubated cold water had concentrations up to 33 ng/L. Hot water had clearly higher levels of BPA in two sites, even up to 1,65 µg/L, whereas in three sites BPA levels were below 60 ng/L. BPF, NP, and OP were detected only in few samples below 2; 1,4; and 22 ng/L, respectively. BPA in tap water samples mainly originates from the epoxy lining, but the levels are low in cold water. In hot water BPA levels were relatively high in 2 houses which were also the oldest studied renovation sites. Possible during time epoxy lining is becoming eroded especially at the hot water line, causing elevated BPA concentrations.

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A novel fractionation approach using four columns in parallel for effect-directed analysis of antiandrogenic compounds in a river water extract

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Within the past decade a number of studies correlated reproductive disorders in humans and wildlife with endocrine disrupting compounds. The focus of research was on chemicals acting as anti-/estrogens. Moreover, there is an increasing evidence for the impact of antiandrogens on this kind of disorder such as the occurrence of feminized fish in river water that has been associated not only with estrogenic but also antiandrogenic compounds. Thus, the aim of this study is the identification of so far unknown antiandrogens by effect-directed analysis (EDA) in the extract of a small, wastewater-impacted river in Germany. The success of EDA is essentially influenced by the fractionation procedure. This study introduces a novel fractionation approach using four orthogonal columns in parallel as a time-efficient alternative to the classical, sequential fractionation scheme. Chemical and toxicological analysis is performed on fractions from the different separation systems. The candidate peaks common in bioactive fractions are selected and subjected to toxicant identification. For the selection of suitable LC stationary phases 52 known or suspected androgens and anti-androgens were separated on 17 different stationary reversed-phases owning widely differing chemistries. The retention data were analyzed using several procedures including principal component analysis, spearman rank correlation of the retention time plots and the approaches according to Gilar et al. and Camenzuli. An aminopropyl-, octadecyl-, pyrenyl ethyl and additionally according to the promising results in the literature a pentafluorophenyl phase were selected. The antiandrogenic activity of the fractions was analyzed using a miniaturized anti-AR CALUX assay due to the limited sample amount at a non-cytotoxic concentration range. One single active fraction was observed for each fractionation with the four selected columns. Non-target screening of the active fractions by use of high resolution mass spectrometry is currently being performed.

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Determination of eleven thyroid hormones and metabolites in plasma and tissue: description of analytical method and ecotoxicological case studies

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Thyroid hormones, such as thyroxine (T_4) and 3,3',5-triiodothyronine (T_3), are vital in numerous physiological processes (e.g. embryonic development, metabolism, cell differentiation and proliferation, cognitive development, and thermogenesis). However, little is known regarding how thyroid hormones affect stress regulation and behavior. Many factors control circulating levels of the bioactive hormone (T_3), consequently not only T_4 and T_3 measurements are vital, but also so-called inactive thyroid hormone metabolites are necessary for a comprehensive description of homeostasis. Circulating thyroid hormones in plasma are typically in low ppt-levels, and can be used as a diagnostic tool during e.g. pregnancy, or for hypothyroidism, hyperthyroidism and endocrine disruption diagnosis. A prerequisite for investigating thyroid hormone disrupting effects is a need for establishing highly sensitive analytical methods. In the present work, we describe an isotopic-dilution LC-MS/MS methodology to determine eleven thyroid hormones and metabolites in 'pico-gram' levels in plasma and tissue from wildlife. The protein-unbound fraction of hormones is largely recognized as the circulating 'bioavailable' fraction. Consequently, free and total thyroid hormone concentrations in blood and plasma are reflected. Finally, we apply the developed methodology to investigate thyroid hormone levels in individual tadpoles (*Xenopus laevis*) ranging from NF stages 55-61 and in plasma from adult *X. laevis*, both from controlled in-vivo studies, and in wildlife samples (e.g. whale, fish, and amphibian).

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Enantiospecific disruption of human steroidogenesis in vitro by the azole fungicide imazalil

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Increasing evidence exists that several pesticides are likely to disrupt hormone homeostasis in humans and may contribute to endocrine related diseases like infertility, obesity and different types of cancers. However, although many pesticides are chiral, hardly any studies exist elucidating how the endocrine disrupting (ED) potential depends on the enantiomeric form. This information is needed for a more precise risk assessment of chiral pesticides and for the development of safer pesticides by for example eliminating the toxic enantiomer

from the pesticide formulation. An important target of various ED chemicals is steroidogenesis, i.e., the synthesis of steroids such as progestogens, sex steroids (androgens and estrogens), and corticosteroids, which control the complex physiological processes associated with growth, reproduction, and pregnancy. A group of pesticides shown to disrupt steroidogenesis are azole fungicides. In this project we analyzed *in vitro* the disruption of human steroidogenesis by the chiral azole fungicide imazalil in dependence of the enantiomeric form. As model system we used the human adrenocortical carcinoma cell line H295R, which expresses all important human steroids. Pure enantiomers of imazalil were isolated with enantioselective HPLC and the absolute configuration of the enantiomers was identified with circular dichroism. H295R cells were exposed for 48 h to racemic imazalil as well as to the individual enantiomers in concentrations ranging from 0.001 – 8 μM. After exposure 17 steroids were quantified in the cell medium using HPLC-MSMS. Racemic imazalil inhibited the synthesis of several steroids belonging to androgens, estrogens and corticosteroids with EC50s in the range of 0.018 – 1.1 μM. First results show that imazalil enantiomers differ in their disruption of some steroidogenic pathways: *S*-imazalil inhibited estrone production approx. 7 times stronger than *R*-imazalil, whereas progesterone production was stimulated by *R*-imazalil to a 3 times higher extent than by *S*-imazalil. These results indicate that the disruption of steroidogenesis by azole fungicides is enantiospecific *in vitro*. Which significance our findings have for the risk of endocrine related diseases has to be tested in future *in vivo* studies.

Science based strategies for the environmental assessment and management of pharmaceuticals and veterinary medicines

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Industry Approach to Managing Potential Risks from Active Pharmaceutical Ingredients in Manufacturing Effluent

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To address stakeholder concerns with pharmaceuticals in the environment (PiE), the pharmaceutical industry has developed a proactive product stewardship approach entitled, ‘Eco-Pharmaco-Stewardship’ (EPS) consisting of three ‘pillars’, namely; enhanced environmental risk assessment (eERA), extension of the scientific knowledge base (IMI-iPiE), and manufacturing effluent management, the focus of this presentation. Through the application of a risk-based approach, the industry is seeking to ensure that manufacturing effluent discharge is effectively managed to minimize environmental risk across the supply chain. The development and application of step-by-step guidance by several manufacturers will be described, as well as, steps taken to ensure all in the supply chain are aware of practices that can be deployed to ensure that risks from manufacturing effluent are low. The use of a maturity ladder concept to establish current program sophistication level and gauge advancement will also be presented.

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The need for targeted testing to improve the regulatory environmental risk assessment of veterinary medicines used in aquaculture

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The use of pesticides to treat farmed salmonids for infestations of salmon lice has increased significantly over the recent years. Controlling salmon lice infestations is not simply an economic goal, but also it is a requirement that fish farms maintain levels below 0.1 lice per fish to limit transmision to wild poulations of salmonids. With the emergence of more resistant strains of salmon lice, control strategies are becoming more aggressive and the consequence to non-target organisms is currently receiving a great deal of media publicity. For some pesticides used to treat salmonids, standard acute toxicity tests may be sufficient to predict environmental effects on non-target organisms exposed to veterinary medicines in aquaculture. However, standard acute toxicity tests are not sufficiently protective to estimate possible environmental effects of substances that have a very specific mode of action, for example chitin synthesis inhibitors. Therefore, more targeted assessment strategies using non-standardised test methods aimed at the specific mode of action is necessary to capture the possible long term effects of these pesticides. This presentation describes some of the pesticides that are currently being used to treat salmon lice and the possible environmental impact that they may be having on non-target organisms plus recommendations for improvements to the environmental risk assessments (ERA) of veterinary medicines used in aquaculture.

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Refined exposure estimation to support an Environmental Assessment for a veterinary medicine

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The U.S. Food and Drug Administration, Center for Veterinary Medicine, evaluates whether significant environmental impacts would occur with the approval of new animal drugs pursuant to the National Environmental Policy Act. The approval process may require the preparation of an environmental assessment (EA), which contains sections addressing problem formulation, environmental fate, exposure, effects and risk characterization. Using a recent EA as a framework, this presentation will focus on the refinement of environmental exposure estimates using spatial techniques to identify representative and protective environmental scenarios, and link them to exposure models commonly used in the U.S. Environmental Protection Agency (USEPA) pesticide registration process. A Geographic Information System (GIS) was used to identify regions of high exposure potential across the US based on beef cattle characteristics and climatic conditions. From within each region, a single vulnerable watershed was selected and characterized for watershed-scale modelling following USEPA Tier-2 pesticide exposure approaches. Three potential sources of chemical were modelled: feedlots, agricultural fields applied with manure collected from the feedlots, and pasture. Using PRZM and EXAMS models, runoff and erosion inputs to surface water from these sources were assessed over a 30-year timeframe to produce final PECs suitable for use in the effects portion of the EA. The results of the national vulnerability assessment identified five regions with diverse intensive-use characteristics. From within these, a single intense-use watershed was selected and modelled. Loadings from each of the land covers were combined on a daily basis and transported to the receiving water body, from which daily PECs were calculated. Based on the aggregate aquatic exposure, no significant effects were identified and a Finding of No Significant Impact (FONSI) was determined. The process presented here discusses the development of refined methods to estimate exposure using spatial techniques to identify representative and protective environmental scenarios. It linked these scenarios to accepted EPA exposure models which addressed all potential sources of chemical loading and produced a series of surface water PECs suitable for risk characterization. This approach is a robust and viable methodology incorporating real world information but maintains inherent safety assumptions from USEPA Tier-2 pesticide framework.

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Use of Acute and Chronic Ecotoxicity Data in Environmental Risk Assessment of Pharmaceuticals

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For many older pharmaceuticals, chronic aquatic toxicity data are limited. To assess risk during development, scale-up and manufacturing processes, acute data and physicochemical properties need to be leveraged to reduce potential long-term impacts to the environment. Aquatic toxicity data were pooled from daphnid, fish and algae studies for 102 active pharmaceutical ingredients (APIs) to evaluate the relationship between predicted no-effect concentrations (PNECs) derived from acute and chronic tests. The relationships between acute and chronic aquatic toxicity and the *n*-octanol/water distribution coefficient were also characterized. Statistically significant but weak correlations were observed between toxicity and log Dow, indicating Dow is not the only contributor to toxicity. Both acute and chronic PNEC values could be calculated for 60 of the 102 APIs. For most compounds, PNECs derived from acute data were lower than PNECs derived from chronic data, with the exception of steroid estrogens. 7% of the PNECs derived from acute data were below the EU action limit of 0.01 μg/L and all were anti-infectives affecting algal species. 8% of available PNECs derived from chronic data were below the EU action limit and fish were the most sensitive species for all but one API. These analyses suggest that the use of acute data may be acceptable if chronic data are unavailable, unless specific mode of action concerns suggest otherwise.

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Evaluating the Risk of Pharmaceuticals in the Terrestrial Food Web

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This study investigated the risk to bird species consuming earthworms contaminated with active pharmaceutical ingredients (APIs) using a combination of previously published earthworm bioconcentration factors (BCFs) and new experimental results. Food chain transfer scenarios were subsequently compared between soil - earthworm - bird using a range of pharmaceuticals. In total, an exposure assessment was carried out to evaluate food web transfer of selected APIs (carbamazepine, diclofenac, fluoxetine and orlistat) through 28 different field scenarios, taking into account the effect of soil type and species traits on earthworm API uptake. API residues were predicted in two earthworm species, namely *Eisenia fetida* and *Lumbricus terrestris* from experimentally determined BCFs and environmentally relevant soil concentrations. Using a combination of calculated API residues in earthworms together with known consumption data of earthworms for the selected bird species, the daily intake of each API by song thrush, rook, starling, lapwing and common gull was modelled. Based on these exposure scenarios the resulting earthworm concentrations reached a maximum of 2462.03 μg/g. Subsequent analysis demonstrated that the food web transfer of selected pharmaceuticals reached a maximum daily intake of 459.8 μg per bird. Ultimately, a range of parameters can influence the bioavailable fraction of the API in the soil which can lead to a range of bird daily intake values. Sandy soils typically have higher bioavailability than clay soils which can influence earthworm API uptake and thus bird exposure. In general, the larger BCFs for *L. terrestris* resulted in a higher uptake in comparison to *E. fetida*, given a fixed soil concentration, and thus a greater intake by the bird species on a per g basis. To evaluate any potential long term toxicity by birds ingesting API contaminated earthworms the bird elimination half-life required to accumulate the human therapeutic dose (HTD) in 90 days was subsequently calculated. The bird species included in this analysis would require elimination half-lives in the range of 1 to 3 orders of magnitude longer than humans to accumulate the HTD. Therefore, as birds typically have faster metabolic rates than humans, and so are likely to eliminate APIs quicker than humans it is unlikely that the calculated elimination half-lives required to accumulate the HTD would be observed.

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Post-approval environmental management of human medicinal products: An extended environmental risk assessment (eERA) framework
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The Association of European Self-Medication Industry (AESGP), European Federation of Pharmaceutical Industry Associations (Efpia) and the European Generics Association (EGA) are currently looking to implement a holistic approach to the environmental management of human medicines products called EcoPharmacoStewardship (EPS). Within this EPS initiative, AESGP, Efpia and EGA propose to extend the ERA paradigm into the post-approval phase (specifically for new APIs) to refine usage figures based on total substance use and investigate and report environmental concerns identified post-approval. Should the ERA outcome change as a result of these eERA activities, risk management options could be discussed with stakeholders and appropriate measures may be agreed by participants. Through three distinct phases, there are several benefits that eERA brings to the environmental assessment of human medicinal products containing new APIs. These include: (i) formalising post-launch commitments for addressing environment risk without impacting patient access to medicines, (ii) a risk assessment based on the total PEC arising from all products containing the same API, (iii) on-going assessment of the relevance and reliability of research findings, (iv) updating the ERA where necessary, (v) agreeing with regulatory agencies what follow up risk refinement and risk management measures that may be required, and (vi) determining the likelihood that any research findings can translate to an adverse impact in the wild. eERA offers a possible mechanism where industry can agree with the EMA and other stakeholders (including national competent authorities (NCAs)) on proportionate risk management measures where any significant environmental risks identified post-patient use would trigger appropriate further work to refine the ERA. Conversely, where post-authorisation surveillance does not indicate any significant risk for an API then no further action is needed until the next scheduled review of the ERA based on Total PEC. This presentation will describe the eERA process, its benefits, and discuss some of the challenges posed in its implementation.

Advancements in life cycle impact assessment method development (II)

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Assessment of the impact of pesticide application in agricultural LCA: sensitivity analysis of PestLCI and USEtox
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The assessment of fate and ecotoxicity of pesticides within a Life Cycle Assessment (LCA) is a challenging task. On one hand the number and possible ecotoxic effects of the different active ingredients is high. On the other hand modelling the fate of a chemical substances in the environment is not straightforward regarding the complex interdependencies of the various processes. Current modelling of the fate of pesticides after their application in the Life Cycle Inventory (LCI) and the subsequent Life Cycle Impact Assessment (LCIA) in LCA studies and databases proved to be insufficient, since the fate of pesticides was not modelled in the inventory phase. In this analysis we scrutinized the two following models with improved bio-physical modelling: PestLCI and USEtox. PestLCI tackles the fate modelling of pesticides immediately after application, while USEtox provides characterisation factors (CF) assessing the impact of a pesticide in different environmental compartments. These two methods have been increasingly used in combination in recent LCA studies of agricultural production systems. In order to assess the practicability of PestLCI and USEtox for the calculation of agricultural LCA and to guide data collection we conducted a sensitivity analysis. The goal was to a) identify important input parameters, b) draw conclusions for the required precision of the input data, and c) to estimate where default values could be used. In the sensitivity analysis nine input parameters were independently varied according to prevailing conditions in Swiss agricultural practices. In line with previous sensitivity analyses performed with PestLCI, we found that soil and climate are critical parameters. In addition we found that the development stage of the crop, tillage, buffer zones, and drainage are further important input parameters. Low sensitivity of results was found for the input parameters irrigation, tillage, and share of macropores. Similar results were found in an additional sensitivity study where the impact score (IS) consisting of results from PestLCI and USEtox was assessed. The presented results suggest that further research is needed to define the soil and climate profiles that are requiered to cover the main agricultural areas. On the other hand we identified input parameters with low sensitivity where default values can be used. These findings help to structure and simplify the data acquisition and calculation of the impact of pesticides within a LCA.

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Synergies and divergence between LCA human toxicity assessment and Risk Assessment approaches
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1. Introduction Beyond the assessment of toxic effects and risks within the framework of European regulations, such as REACh and CLP, other assessment methods for sanitary risks exist but differ from one country to another. Methodologies are different but always based on the founding principles of dose-response relationships and exposure scenarios describing the source-to-target vector. The complementarity of Life Cycle Assessment (LCA) and Chemical Risk Assessment (RA) may yield a more accurate and exhaustive approach to assess human toxicity. 2. Materials and methods 2.1. Panorama of methods assessing human toxicity Various approaches to assess human toxicity risks exist and allow to supply toxicity data. The classifications, such as those established by the European Union, allow to identify hazards of substances. Risk and hazard based approaches typically apply (reasonable) worst-case assumptions for modelling and data selection. In contrast, LCA toxicity assessment methods apply the concept of best-estimates. Another important difference is that RA is site-specific whereas LCA is site-generic. A number of relevant methods for human toxicity assessment within the LCA and RA frameworks have been analysed in order to allow for a mapping of the methods. The results and the divergences identified between the methods are presented in a condensed way. 2.2. Complementarities The methods have been analysed according to many criteria. This talk will underline the similarities and differences as well as the advantages and the associated drawbacks linked to each method. To support this analysis, a comparison based on a case study applying both LCA and RA methods was performed for the human toxicity assessment of a paraben-free cosmetic formula. A detergent was also analysed with different methods. Throughout the study, independent scientific experts have been associated to assure a peer-review of this study. 3. Results and discussion Propositions for the correct interpretation of results as well as their limitations and research needs were identified. Methodological issues will be discussed. Moreover, guidelines and recommendations for human toxicity assessment will be proposed, detailing which method serves which purpose, where they overlap and where they complement one another. This study invites experts to work together to find solutions to the current issues in human toxicity assessment.

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Addressing fresh water ecotoxicity impact category under the EU Product Environmental Footprint (PEF)
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The UNEP-SETAC Life Cycle Initiative recommended LCA method to assess the impact of chemicals on the aquatic environment and human is the USEtox model.

USEtox is also the EU Commission recommended method to calculate 'Ecotoxicity for fresh water' and 'Human toxicity' impact categories in the context of the EU Commission Product Environmental Footprint (PEF). This model is now considered by the LCA community as the most consensual for estimating the impact of chemical emissions of human health and aquatic ecosystems. Since its release, USEtox has been widely used by research organizations and consulting firms, but only very recently, in the context of the PEF, this model has been systematically used and evaluated by several sectors of industries for the purpose of product comparison and communication. This 'real life testing phase' has revealed some issues that required immediate attention if the result of this model is to be used for hot spot analysis, product improvement, product comparison and communication. In January 15th 2015, the EU Commission has organized a workshop with all the PEF pilots that have used the USEtox model in their screening studies. The main conclusions from this meeting were: The experience of using the USEtox model by all pilot members was somehow disappointing. The model was not criticized as such, but rather the outcomes of the calculation (i.e characterisation factors (CFs)). The lack of transparency as well as complexity of using the model to calculate new characterisation factors were identified as a limitation for further use. The data (physico-chemical, half-life and toxicity) that have been used to calculate the CFs provided with the model (about 3000) seems to be a source of significant differences, controversies and criticisms. For many substances, especially fate and effect data may not be suitable as currently used in USEtox. Most PEF Pilots recommended not to use the model further before agreement is reached on the selection of input data After this workshop, the Joint Research Centre (JRC-Ispra) has conducted an in-depth evaluation of the model and data used to calculate CFs. This paper aims at highlighting the main areas where modifications could be made to improve the reliability and acceptance of the CFs

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Analysis of the different technique to include noise damage in life cycle assessment. A case study for car tires.
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WHO (2011) reported very comprehensively on the burden of disease from environmental noise, highlighting noise as a threat to public health problem. Despite this need, the integration of traffic noise damages on human health in life cycle assessment (LCA) is still debated. Although several methodological attempts were advanced to fill in this gap, none of them has become consensual so far. The objective of this study is threefold: i) to analyse how the different methods for noise damage assessment are sensitive to different input noise levels; ii) to compare the results obtained from the three methods and iii) to evaluate the importance of noise damages on human health as compared to other burdens contributing to the DALYs. To this aim, three methods for noise damage assessment are investigated. A case study on car tires is considered as seminal example of functional unit to highlight and discuss the differences between the results obtained from the three methods. For our case study, three different tires are considered: one reference tire, and two tires (Tire 1 and Tire 2) with improved acoustical characteristics provided by a tire manufacturer. The complete LCA of the car tires (functional unit: a tire used over one kilometre) was also conducted using the ReCiPe method following the hierarchist perspective and average weighting set (H,A). i)The results show that all the noise characterization methods are sensitive to the differences in the acoustical characterises of the tires. Thus they allow reflecting in a quantitative way the improved sound level performance of the tire into clear impact differentials expressed in DALYs. ii) The dispersion of the results obtained from the methods show the need for further refinement of the assessment methodology to improve the accuracy and consistency of the integration of noise impact in LCA. iii) Despite the variation between the methods used, noise impact represents at least 90% of the total impact of the tires on human health. It highlights the importance of taking into account noise impact in LCA.

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The development of an operational LCIA-methodology with impact categories based on the control variables in the Planetary Boundaries framework
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This study presents a first attempt at an operational LCIA-methodology basing the definition of the impact categories on the control variables as defined in the Planetary Boundaries (PB) framework. The PB-framework introduced a set of biophysical Earth system processes and defined quantitative PBs that have to be respected for Earth to remain in the Holocene state. The concept is attracting a strong interest from industry as companies seek to assess and communicate the environmental performance of their products relative to the PBs. The PB-framework has previously been attempted included in LCA as part of normalization and weighting. The limitations of both attempts are the lack of spatial differentiation for spatially differentiated PBs and the requirement for harmonizing the control variables with indicators already used in life-cycle impact assessment (LCIA). A way to overcome these limitations is to directly use the control variables in the PB-framework as impact categories in LCIA, which is also the objective of this study. This work defines a mathematical framework for a LCIA-methodology where Characterization Factors (CFs) are included for all Earth system processes in the PB-framework, for all substances contributing to effects on the Earth system processes and expressed in the units of the control variables. Except for novel entities and biosphere integrity which are currently excluded from the LCIA-methodology because the former is lacking a planetary boundary metric while a full understanding of the cause-effect chain is missing for the latter. The CFs were estimated by identifying the environmental models needed to model the control variables of the PB-framework and adapting these to fit the LCIA-framework. This work provides a full set of CFs for all the Earth system processes in the PB-framework. The new LCIA-methodology provide additional and complementary insights which cannot be achieved with traditional LCIA-methodologies. The results provide information on the environmental impacts of the assessed products and solves previous problems with approximative links between control variables in the PB-framework and current LCIA impact categories. The new insights can be used for communicating the product's environmental performance and to support definitions of absolute reduction targets relative to the PBs.

Passive sampling of organic micropollutants and toxicity assessment: opportunities, challenges and innovations (II)

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Passive sampling and toxicity profiling: a case study in Rivers Meuse and Rhine, The Netherlands
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The TIPTOP project hypothesizes that time-integrative passive sampling followed by toxicity profiling is a toxicologically and ecologically more relevant and more protective approach for chemical water quality assessment than the assessment based on a comparison between concentrations of predefined individual compounds to their Environmental Quality Standard (EQS). To test this hypothesis, a demonstration study was designed at well-defined WFD sampling sites in the Dutch delta. Time-integrative sampling was performed using partitioning-based and adsorption-based passive samplers. Toxicity profiling was performed using a test battery consisting of in vitro and in vivo bioassays. Currently, all chemistry and toxicity profiles are available. Several approaches are followed to interpret these profiles in a toxicological and ecological context. First of all, the chemical data are used to make a traditional comparison between water concentrations of individual contaminants and their environmental quality standard concentration defined within the WFD. Moreover, the chemical analyses are used to determine the contribution of the analyzed compounds to the observed toxicity and to calculate the potentially affected fraction of species by exposure to these multiple substances (msPAF). Moreover, molar concentrations in the passive samplers are used to estimate to what extent the critical bodyburden for narcotic compounds is reached and to estimate the msPAF for narcotics. Bioassay results are used to benchmark toxicity profiles from river sampling locations to WWTP effluents. In vitro bioassay results are further used to compare to newly derived trigger values, i.e. bioassay responses below which no ecological risk is to be expected. In vivo bioassay results for organisms belonging to six different taxonomic classes are used to derive a species sensitivity distribution (SSD) for each sampling location, which can be used to determine a msPAF value based on bioassay responses measured after exposure to the whole sample, rather than on a limited set of chemical data. Finally, calculated and measured msPAF values are compared to the actual biological monitoring data obtained at the very same sampling locations. The outcome of TIPTOP should ultimately lead to a proposal for a cost-effective approach for chemical water quality assessment.

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Investigating the time-integrative properties of passive sampling for chemical monitoring and application in bioassays
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Regular chemical water quality assessment of surface water is performed by single grab sampling of the water and comparison to Environmental Quality Standard (EQS). A disadvantage is that a grab sample gives only a snap shot of the water quality and may not be representative for compounds with an irregular emission pattern. Application of (time-integrative) passive sampling might be a better strategy, because water is sampled over a longer period and a higher water volume can be sampled. After deployment of the samplers and extraction in the laboratory, these extracts can be used for chemical analysis in the same way as in

the regular monitoring and/or used in bioassays to perform toxicity profiling. Uptake of passive samplers depend on deployment time and water turbulence or flow. The time-integrative properties of sampling was investigated within the TIPTOP project for partitioning-based and adsorption-based passive samplers Silicone-rubber (SR) samplers and adsorption based Speedisk (SD) samplers were deployed in the water for short and long periods overlapping with each other. \n After deployment the samplers were extracted and analysed on targeted chemical analysis with LC-MS and GC-MS, including PAHs and a range of plant protection substances. In addition all extracts were subjected to toxicity profiling The release of Performance Reference Compounds dosed prior to deployment was used to estimate the sampling rate for Silicon rubber samplers which information was also used to estimate the water volume sampled by the speedisk. In the presentation the time integrative properties of the samplers is evaluated as well as the evolution of the results from toxicity profiling.

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In silico prediction of sampling rates for polar organic chemical integrative samplers (POCIS)
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The modelling and prediction of polar organic chemical integrative sampler sampling rates (R_s) for 73 compounds using artificial neural networks is presented for the first time. Molecular descriptors were chosen based on previous retention descriptor models and also a genetic algorithm which shortlisted 24 descriptors from a total of 201 that covered topological, geometrical and physicochemical properties. Each network was validated by testing the predictive ability for an external validation dataset ($n = 6$) of benzodiazepines with the best network based on chromatographic retention molecular descriptors. The genetic selected descriptor network showed an average absolute predictive error of 0.0437 ± 0.02 L d^{-1} which contrasted to the retention descriptor network error of 0.0145 ± 0.008 L d^{-1}. The sum squared errors for the training, verification and test subset were 0.092, 0.062 and 0.121, respectively. The average predicted error for the verification ($n = 11$) and test ($n = 11$) set was 0.03 ±0.02 L d^{-1} of the experimentally determined R_s value. The network was built in triplicate to assess the variability of predictions across networks and in comparison to experimental variance which gave better or similar variance to the measured values. These novel findings indicate the potential of *in silico* predictive tools for R_s determination which represents significantly more economical approach than laborious laboratory based calibrations.

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Performances of the Continuous Flow Integrative Sampler (CFIS) for monitoring organic pollutants in surface waters
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Passive sampling has recently emerged as an efficient alternative to conventional active sampling for monitoring organic pollutants in water. The technique consists in exposing the passive sampler in the water for days to weeks, depending on the sampler, to accumulate the target pollutants. Average concentrations integrated on the exposure period are then determined from the mass of pollutants sequestered in the sampler. The concentration of the target pollutants in the receiving phase allows reaching lower quantification limits than conventional sampling techniques. Moreover, this technique reduces dramatically analytical costs, as it provides realistic average concentrations with a single analysis. A passive sampler is typically composed of a receiving phase and a membrane that separates the receiving phase from the medium. Several passive samplers have been developed for targeting pollutants of different physical chemical properties, and especially different polarities. The Semipermeable Membrane Device (SPMD) has been developed for non-polar organic pollutants such as polychlorobiphenyls (PCB) and polycyclic aromatic hydrocarbons (PAH) in water whereas the Polar Organic Chemical Integrative Sampler (POCIS) targets polar organic pollutants such as pharmaceuticals and polar pesticides. However, depending on the target pollutants, the performances of these passive samplers may depend on the exposure conditions, and especially on flow velocity. We have recently developed an automated, fully submersible, integrative sampling device to monitor both non-polar and polar organic pollutants in different types of water, the continuous flow integrative sampler (CFIS). Unlike other devices, the performances of the CFIS are independent from flow velocity. The performances of the CFIS were assessed and compared with those of spot sampling for monitoring hormones, pharmaceuticals, PCB, PAH, hydrophobic pesticides and plasticizers in the inflow and the outflow waters of a fish farm in Ardtoe (Scotland). Exposure campaigns of 10 days have been started in July 2015, for one year. For each campaign, the CFIS was exposed for 10 days, while spot samples were collected on the first and the last days of the campaign. Analysis results showed better sensitivity of the CFIS and good agreement between the results of CFIS and spot sampling. Therefore, the CFIS proved to be an efficient, sensitive and cost-effective alternative for monitoring organic pollutants in surface waters.

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Application of miniaturized cell-based bioassays for high-throughput effect-directed analysis of passive sampler extracts using microfractionation
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Routine monitoring of drinking water sources for endocrine disrupting chemicals is commonly limited to the determination of known priority substances through chemical analysis. With the ever changing composition of the mixtures of compounds in the environment, other potentially hazardous chemicals remain undetected. Efforts to keep track of the release of potential endocrine disruptors into the environment and to monitor the processes of drinking water purification depend on the ability to determine the presence of these compounds in surface, ground and effluent water. The advent of cell-based reporter bioassays allowed detection of active toxicants in mixtures based on their biological activity. The application of these bioassays together with analytical identification methods in effect-directed analysis (EDA) provides a strategy to detect and identify novel endocrine disrupting chemicals. However, for rapid toxicity assessment of e.g. water samples, EDA suffers from lack of throughput and is regarded as a laborious, time consuming method. To achieve higher-resolution separation and faster analysis on multiple end-points, a high throughput-EDA (HT-EDA) method was developed through application of UPLC based microfractionation in combination with a battery of miniaturized 384-well plate cell-based reporter assays performed in parallel. The applicability of this approach will be demonstrated by analysis of selected extracts from partitioning based silicone rubber (SR) passive samplers and adsorption based Speedisk passive samplers. The application of high-resolution microfractionation together with miniaturized bioassays will open the way to HT-EDA and will emphasize the advantages of the unification of chemical analysis and bioassays in the analysis of environmental samples, including drinking water.

Antibiotics and Antibiotic Resistance in the Environment: Ecological Fate and Effects, Resistance Development and Implications for Human Health

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Co-selection for antibiotic resistance by biocides in the aquatic environment
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Antibiotic resistance is a growing problem in the treatment of clinical infections, leading to predictions that we are about to enter a post-antibiotic era where many bacterial infections are untreatable. Increasing attention is being placed on the environment as a reservoir of antibiotic resistant bacteria and in the potential for antibiotic residues and other compounds to select or co-select for antibiotic resistance. Quaternary ammonium compounds are used as biocides and are found in a wide range of personal care products, reaching mg/Lconcentrations in waste water. Work by Gaze et al. (2005) was the first to demonstrate co-selection by quaternary ammonium compounds (QACs) for antibiotic resistance in the environment. Recent work has shown that high QAC exposure selects for QAC resistance mechanisms that have also been implicated in resistance to antibiotics. Functional metagenomic libraries were screened for resistance to biocides, and transposon mutagenesis used to identify and sequence novel biocide resistance genes. Current work focuses on selection for QAC resistance and co-selection for AMR in experimental microcosms, to determine the minimal selective concentration (MSC) of QACs that co-select for antibiotic resistance. Data suggests QAC concentrations in highly polluted waste streams are in the same order of magnitude as the MSC.

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Selection for antibiotic resistance in the environment
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Antibiotic resistant bacteria are a significant threat to both human health and the global economy. As few new antibiotics are currently in development, antimicrobial stewardship is currently the best strategy to avoid progression into a ‘post-antibiotic era’, a world in which most modern medical and farming practices will be unviable, and routine infections could once again become deadly. Most

mitigation strategies implemented thus far have focused primarily on intervention at the clinical level, and the role of the natural environment has been largely overlooked. However, it is known that the environment is not only a reservoir of clinical resistance genes and that these can be mobilised into human pathogens, but that resistance genes and antibiotics are constantly being introduced into the environment via several sources including waste water treatment plant effluent and run off from agricultural land. A key study by Gullberg *et al*. (2011) demonstrated that selection for resistance can occur at extremely low (potentially environmentally relevant) antibiotic concentrations, and therefore the environment may even be a platform for selection for resistance *in situ*. However, there are currently no standardised methods in practice which can estimate the effect concentrations of antibiotics in the environment, in terms of selecting for, maintaining, or mobilising resistance genes. Therefore mitigation strategies have been unable to be designed. In this study natural, complex communities were exposed to varying concentrations of antibiotics and resistance gene prevalence quantified by real time PCR. Antibiotic concentrations were chemically quantified and antibiotic degradation curves generated to determine the optimal culturing conditions to emulate continuous exposure at clinical and subclinical concentrations. The primary aim of this research is to understand how resistance may be selected for in the environment, and to design an assay which can be used for environmental risk assessment.

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Impact of dairy manure pre-application treatment on dynamics of antibiotic-resistance genes in crop production systems

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Antibiotics are widely used in livestock and poultry production for growth promotion, prophylaxis and treatment of illness. There is a widespread concern that this practice promotes resistance and erodes the efficacy of antibiotics used in human medicine, a crucially important public health challenge. The recycling of animal and poultry wastes into crop production systems to recycle valuable nutrients and improve soil quality is widely practiced. However, a reservoir of antibiotic resistance genes in the environment that is made larger through contamination with agricultural wastes may represent an enhanced threat to human health. In the present study, we sought to determine if pre-treatment of dairy manure through composting, dewatering, or anaerobic digestion would alter the loading rates of antibiotic resistance genes, their persistence in soil following application, and the burden of antibiotic resistance genes on crops at harvest. Experiments were undertaken during the 2014 growing season on the Agriculture and Agri-Food Canada research farm in London, Ontario, Canada. Manure for applications in the spring of 2014 was obtained from two local dairy farms. One farm supplied the raw manure slurry, and anaerobically digested manure digestate. The second farm supplied the mechanically dewatered, and the composted manures. Replicated field plots received no manure (ie. untreated control), raw, composted, dewatered or anaerobically digested dairy manure. Plots were planted to carrots, radish, or lettuce. DNA was extracted from manures, soils throughout the growing season and vegetables at harvest. These were variously analyzed by straight PCR, quantitative PCR, or high throughput sequencing to establish the loading rates, soil persistence, and exposure of vegetables to selected gene targets associated with antibiotic resistance or gene mobility. Composted manure had the lowest abundance of gene targets, whereas anaerobic digestate that the highest. There was no consistent effect of manure pre-treatment on persistence of gene targets in soil following manure application. Referenced to vegetables grown in ground without manure there was no consistent increase in gene target abundance on vegetables grown in manured ground.

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Fate of antibiotics into agricultural soils amended or not with organic waste products, and combined effects of contaminants

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Antibiotics (ATB), widely used in human and veterinary medicine, are for the most part excreted. Waste water treatments do not eliminate them completely. Consequently, spreading organic waste products (OWP) issued from sewage sludge or liquid and solid manures may disseminate ATB in soils with unknown risks for human health and environment. In addition, these OWP contain other contaminants such as trace metals (TM), which tend to accumulate in soils submitted to repeated spreadings. Interactions between contaminants may affect the fate or and the potential effects of ATB.\nOur objectives were to investigate the impact of soil type and combinations of ATB and TM on the degradation of two ATB in soils, ciprofloxacin (CIP) and N-acetyl-sulfamethoxazole (N-SMX). Cu and Zn were chosen as TM. Soil incubations of 14C labelled ATB under laboratory conditions were used to quantify over time their fate and distribution in the soil (mineralized fractions, easily and hardly extractable fractions, and non-extractable fraction).\nThe influence of the type of soils was studied from applications of N-SMX (0.02 mg / kg) or CIP (0.15 mg/kg), on three agricultural soils likely to harbour a microflora more or less adapted to degradation of ATB: a control, unamended soil and two soils amended every two years since 1998 respectively with a compost of sewage sludge/green waste and with farmyard manure. Results showed that the N-SMX mineralization was low, in the order of 10% after 150 days, whereas mineralization of the CIP was negligible. The majority of the radioactivity was concentrated in the hardly extractable and non-extractable fractions. The fate of these ATB has been barely affected by soil type.\nThe impact of combined inputs of N-SMX and TM on the fate of N-SMX was studied through applications of N-SMX alone (0.02 mg/kg) in the control soil, or in a solution of copper and zinc, at realistic doses (respectively 20 and 30 mg/kg) and at five times this dose (respectively 100 and 150 mg/kg). The presence of metals provided at the highest dose resulted in a decrease in mineralization and an increase in adsorption, attributed to a direct effect of metal (complexation with N-SMX and inhibition of the activity of certain microorganisms) and to an indirect effect via a decrease in pH.

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Adsorption and ecotoxicity of three pharmaceuticals: cocktail effect

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Pharmaceuticals are a class of emerging contaminants whose production has increased for many years andwhich are emitted in large amounts to the environment *via* different pathways (wastewater treatment plants effluents, sludges, manure…). They are continuouslydetected in the environment because their rate of removal is compensated by the daily input of new molecules, and they present a risk of toxicity for non-target organisms and human health. In the environment, they are present as mixtures of compounds and they coexist with other pollutants such as, for example, metallic trace elements. Nowadays, there is a lack of information about their behaviour when present in mixtures and about the potential interactions between the different compounds (cocktail effect).Indeed, metals canfor example interact with pharmaceuticalsin particular throughthe formation of complexes which may affect theirbehaviourin the environment. Among the processes governing the fate of pharmaceuticals, adsorptionprocesses play a key role controlling their mobility and bioavailability to organisms in soil and water compartments. It is thus necessary to bring knowledge about their behaviour at solid/water interfaces and their toxicity towards organisms considering the cocktail effect in order to design more realistic scenarios. The aim of this work is to bring knowledge about the behaviour in the environment of three pharmaceuticals considering their cocktail effect: sotalol (a beta-blocker), furosemide (a diuretic) and sulfamethoxazole (an antibiotic).We present in this study (i) their adsorption behaviour on a selected soil as a function of concentrations, which is needed to define the mobility of the compounds and to precise at which extent they are available to organisms, and (ii) their toxicity on two aquatic organisms (*Vibrio fischeri*, and *Daphnia magma* at different concentrations. In each case, we studied the behaviour of each molecule taken separately and of mixtures containing the three pharmaceuticals. In addition, the influence of the presence of copper(II), an ubiquitous metallic cation, was also considered. **Keywords**: adsorption, toxicity,pharmaceuticals, metals.

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Occurrence and environmental risk assessment of selected antibiotics and antiretroviral drugs in Nairobi River Basin, Kenya

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In this study, we investigated the occurrence of three antibiotics (sulfamethoxazole, trimethoprim and ciprofloxacin) and three antiretroviral (lamivudine, nevirapine and zidovudine) drugs in Nairobi River Basin, Kenya. The analytical procedure involved extraction using solid phase extraction followed by liquid chromatography-electrospray ionization tandem mass spectrometry (SPE-LC-ESI-MS/MS). In the study, 40 sites were selected for sampling, including 38 sites along the rivers and 2 wastewater treatment effluents sites. All the studied compounds were detected with sulfamethoxazole having the highest detection frequency of 97.5% and ciprofloxacin had the lowest at 60%. The results showed that the concentration of the drugs increased in highly populated regions especially within the informal settlements. The maximum (median) concentration in the river waters for sulfamethoxazole, trimethoprim, ciprofloxacin, lamivudine, nevirapine and zidovudine in ng/L were 13800 (1800), 2650 (327), 509 (129), 5430 (1000), 4860 (769), and 7680 (660), respectively. The maximum concentrations in the river waters were generally higher than those of the wastewater treatment plant effluents signifying that the rivers are substantially contaminated by domestic wastewater. The environmental risk was evaluated by calculating the risk quotients (RQs) for algae, daphnia and fish based on the maximum and median concentrations of the analytes in the river basin and was expressed as the ratios of measured environmental concentrations (MEC) to predicted no effect concentrations (PNEC). The RQs ranged from 0–507.8 and apart from lamivudine that had a low RQ, all the other analytes had RQ>1 at maximum and median measured concentrations for at least one taxonomic group. The high RQs are indicative of possible adverse ecological effects and calls for corrective and mitigation strategies. Key words: Antibiotics, antiretroviral, occurrence, risk

Pushing nanoparticle studies to the limit - working at environmentally relevant concentrations and with complex matrices (III)

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Changes in the metabolome of Mytilus galloprovincialis exposed to fullerene aggregates under environmentally relevant conditions

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The environmental study of nanomaterials has been a thriving topic during the last years because of their high number of potential emission sources; unique transport and distribution patterns, different to those of the conventional micropollutants; and the high uncertainty around their ecological effects. Among them, fullerenes have received a particular attention and their presence in rivers [1,2], soils [3], wastewater effluents [4] and air particulate [5] has been recently characterized. Because of their particular colloidal behaviour the properties and toxic effects of fullerenes in experiments at lab-scale are highly influenced by the dispersion method and physico-chemical properties of the medium. Therefore, conducting experiments simulating conditions similar to those observed in the real environment is crucial in order to create aggregates with realistic toxic effects and results that are translatable to the real environment. In the present work fullerenes were dispersed and aged in artificial estuary water (with controlled ionic strength, pH and humic acid content) by means of long time stirring with air and sunlight exposure. The suspensions were characterized by means of scanning electron microscope and nanoparticles tracking analysis. The obtained suspensions were employed in metabolomic experiments involving Mediterranean mussels (*Mytilus galloprovincialis*). The exposition was carried out in marine mesocosms of 450 l with pristine seawater at sub-ppb concentration levels. The metabolomic profile of the exposed mussels was analyzed by LC-ESI-HRMS with hybrid qudrupole-Orbitrap analyser in data-dependant scan acquisition mode. Several time-dependant changes were observed in the tested organisms, including changes in the lipid composition, changes in the aminoacids profile and the appearance of oxidative stress biomarkers, such as ophthalmic acid. Overall, the results are consistent with an oxidative stress response, which is observed even at sub-ppb concentrations at metabolic level and that raises questions about the chronic toxicity of these emerging contaminants. [1] J. Sanchí *et al*. (2014) *Analytical and bioanalytical chemistry*, *407*(15), 4261-4275. [2] A. Astefanei *et al*. (2014) *Journal of Chromatography A* 1365, 61-71. [3] J. Sanchís *et al*.(2015) *Science of the Total Environment*, *505*, 172-179. [4] M. Farré *et al*. (2010) *Journal of hydrology*, *383*(1), 44-51. [5] J. Sanchís *et al*. (2011) *Environmental science & technology*, *46*(3), 1335-1343

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Assessing the heteroaggregation of manufactured nanoparticles with naturally occurring colloids in a typical surface water

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To assess the risk posed by nanotechnology-enabled products, the likelihood of engineered nanoparticle (NP) exposure through aqueous media must be considered, as it is a receptacle of these materials throughout their lifecycle. The predicted concentrations of engineered NPs in surface water systems are expected in the μg/L level for nano-TiO_2, and consequently, in real aquatic systems, the probability that NPs interact with each other may be lower relative to their collision frequency with naturally occurring colloids present at substantially higher concentrations (mg/L to g/L). Due to the high specific surface area and reactivity of colloids, they may strongly affect the fate and transport of NPs via heteroaggregation processes. Thus, fate models aimed at predicting NP behaviour and concentration profiles must account for this heteroaggregation. A NP-colloid sticking efficiency, α_{hetero}, is well suited as an input for such fate models, but remains a challenge to determine experimentally. Here, we present a novel method for determining α_{hetero} at environmentally relevant NP concentrations by using a combination of laser diffraction measurements and aggregation modeling based on the Smoluchowski equation. Interactions between TiO_2 NPs (15 nm) and different types of larger mineral colloids (i.e., silica microspheres, smectite clay, and natural riverine suspended particulate matter) were used to demonstrate this new approach. Studies were conducted at low NP concentrations (0.1 to 4 mg/L) with regard to the colloid occurrence (100 mg/L) to develop realistic fate scenarios for surface water systems. The NP/colloid number ratio was found to be a critical component in the heteroaggregation mechanism and the effects of ionic strength, pH, and natural organic matter on NP heteroaggregation were also explored. Our data show that at relevant concentrations, NP behaviour is mainly driven by heteroaggregation with colloids, while homoaggregation remains negligible. The dimensionless α_{hetero} value is a key parameter needed to feed environmental fate models that are of high importance in the field of risk assessment of engineered NPs, as they contribute to better predict the exposure aspect in aqueous systems with continuously increasing relevance. Work funded by the French National Research Agency as NANOHETER program under the frame of SIINN, the ERA-NET, and the EREF. France – U.S. bridge funded by the PUF Program. http://nanoheter.cerege.fr

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Effect of surface coating on nanoparticle stability and fate in high strength electrolytes - silver nanoparticles in marine waters

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Much of the early work on the characterisation of nanomaterials focused on pristine nanomaterials in simple matrices such as ultrapure or freshwater. Characterisation of nanoparticles in more complex matrices such as physiological fluids has not had the same level of attention paid to it and analysing nanoparticles in such matrices remains a significant challenge. While some methods for the extraction of nanoparticles from confounding matrices have been brought to bear, they are relatively harsh leading to the nanoparticles or their (bio)surface coating being modified as a result of the extraction process. Hence, the characteristics of extracted nanoparticles that are ultimately determined are not the same as when they were originally in the matrix. This has a critical impact on toxicity studies as these extracted nanomaterials are ultimately only proxies for the original biological identity of the nanomaterial that would have been encountered by living organisms. To investigate the role of a bio-corona in a complex matrix, we have studied bovine serum albumin-coated silver nanoparticles as a proxy for probing the impact of a bio-coating on nanoparticle behaviour and fate in high strength electrolyte solutions. We have found that bovine serum albumin (BSA) stabilizes silver nanoparticles in high strength electrolytes, with the stabilisation effect increasing with both ionic strength and BSA concentration. The ion release kinetics of the dissolution of silver nanoparticles is significantly lower in the presence of BSA and may be related to BSA suppressing ion release or acting as a store for silver ions and hence the measured quantity of ions released in solution is misleading. Thus understanding the impact of a bio-corona on nanoparticle behaviour in complex matrices is a key preliminary factor that must be considered when modelling nanoparticle fate and investigating their toxic potential in real life scenarios.

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Extrapolated long-term stability of titanium dioxide nanoparticles and multi-walled carbon nanotubes in artificial freshwater

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The potential environmental release and the resulting exposure to engineered nanomaterials (ENM) along their life cycle are expected to constantly increase (Nowack et al., 2012; Pettitt and Lead 2013). Based on environmental exposure scenarios, water is one of the main compartments where ENMs can be released (Nowack et al., 2014). Similar to colloidal particles, upon release into the environment, intrinsic physico-chemical properties of ENMs as well as the specific environmental conditions mainly determine fate and behavior of ENM (Hartmann et al., 2014). Therefore, a comprehensive characterization of all the processes affecting the ENMs system stability is needed. Among all, transformation processes, such as surface modification, homo- and hetero-aggregation, and transport processes, i.e. advection and sedimentation, have been identified as key factors to affect fate and behavior of ENMs in the aquatic environment (Quik et al., 2014). In this work, long-term stability of ENMs, i.e. the inorganic n-TiO_2 P25 and the organic Multi-Walled Carbon Nanotubes (MWCNTs) NC7000, dispersed in artificial freshwater (5-100 $mg \cdot l^{-1}$), was investigated by extrapolation from short-term stability experiments. Hydrodynamic diameter and ζ-pot, calculated by means of Dynamic and Electrophoretic Light Scattering, respectively, qualitatively indicated a general ENMs dispersions instability over 1 h time. Sedimentation results, obtained by Centrifugal Separation Analysis using the LUMiSizer over approx. 30 min of analysis, allowed to estimate the quantitative long-term (over 30 days) stability of ENMs. Settling data fitted satisfactorily with a first order kinetic equation (R^2 in the range of 0.918-0.989). The extrapolated settling rate constant *k* values at gravity spanned one order of magnitude, i.e. from $7.21 \cdot 10^{-5}$ to $4.12 \cdot 10^{-4}$, and increased with the increasing of initial ENMs concentration. Sedimentation velocities were in good agreement with those reported in literature ($7.8 \cdot 10^{-2}$ - $1.7 \cdot 10^{-1}$ $m \cdot d^{-1}$ vs. $5 \cdot 10^{-4}$ - $3 \cdot 10^{-1}$ $m \cdot d^{-1}$ for n-TiO_2 and $5.9 \cdot 10^{-2}$ - $3.4 \cdot 10^{-1}$ $m \cdot d^{-1}$ vs. $2 \cdot 10^{-1}$ – 1.2 $m \cdot d^{-1}$ for MWCNTs). n-TiO_2 showed a higher long-term stability with respect to MWCNTs (average: $1 \cdot 10^{-1} \pm 3.4 \cdot 10^{-2}$ $m \cdot d^{-1}$ instead of $1.7 \cdot 10^{-1} \pm 1.1 \cdot 10^{-1}$ $m \cdot d^{-1}$, respectively).

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Assessing the detection limits of multi-isotopically labelled CdSe/ZnS quantum dots in natural and biological environments

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The vast majorities of NPs-related studies presented in the literature are carried out in conditions far from those in real environmental medium [1] (water, soil, sediment). In particular, the high concentrations (>1 mg/L) of nanoparticles (NPs) used in experimental studies are related to the difficulty of detecting nanoparticles in complex and "noisy" natural and biological environments. However, changes in NPs concentration can affect its physicochemical behavior (ex: solubility, aggregation) and thus the final interpretation and understanding of results. In order to overcome analytical barriers while working at representative realistic concentration, innovative tools such as HR-ICP-MS and non-traditional stable isotopes (isotopically modified NPs [2] or "spiked") have been used. 7 nm sized isotopically labelled quantum dots (QDs), CdSe/ZnS core-shell structure were synthesized, enriched in ^{68}Zn, ^{77}Se and ^{111}Cd. They were disseminated at very low concentrations (0.1 ng/L to 5 μg/L) in both aquatic and biological matrices and then analyzed by HR-ICP-MS (ThermoScientific Element II) based on the protocol described by Dybowska et al. [3]. Preliminary results allowed to assess the detection and quantification limits of spiked QDs in complex matrices such as river water or saliva and plasma. The feasibility of isotopic labeling at very low concentrations has been demonstrated: spiked Zn, Cd and Se issued from QDs were quantifiable at 10, 3 and 20 ng/L respectively in a media not containing the same natural elements, and at 1000, 150 and 1000 ng/L in saliva, and at 50 ng/L of spiked Zn in Seine river water. The results obtained in this experimental work are applicable for studying QDs fate and behavior in most aquatic and biological media.

Fish model species in environmental toxicology

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Addressing whether roach in English rivers have adapted to the harmful effects of exposure to oestrogenic wastewater treatment work effluents

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Wastewater treatment work (WWTW) effluents comprise a large proportion of the flow of lowland rivers across the world. Many WWTW effluents are oestrogenic and induce a range of feminised phenotypes in wild male fish including the intersex condition – the presence of developing eggs in the testes of otherwise male fish. In UK rivers, the reproductive success of the most feminised male roach (*Rutilus rutilus*) is reduced by up to 76%. However, population genetic analyses have identified some populations of roach that have been largely restricted to stretches of river with a high proportion of oestrogenic effluent over multiple generations. This raises the question of whether roach can, and have, adapted to the harmful effects of exposure to oestrogens. To investigate this, we are searching for footprints of selection by analysing single nucleotide polymorphisms (SNPs) in wild roach populations; in tandem with an experimental approach, to examine differences in oestrogen sensitivity. We assembled a transcriptome for roach and used it to identify SNPs in a suite of oestrogen-responsive genes including aromatases, oestrogen receptors and vitellogenins. Genotyping wild roach populations derived from four effluent contaminated rivers (Lee, Foss, Aire and Mole) and five other river stretches that receive little or no effluent using this SNP panel revealed variation in oestrogen-responsive genes, and we are currently investigating patterns of selection in these genes. Analysis of SNPs derived from restriction-site associated DNA (RAD) tag genotyping of roach from the polluted River Lee and nearby clean river Cuffley Brook identified diverse genes with signatures of selection including several that are related to regulation of cell growth and DNA repair in humans. Functional annotation in DAVID identified two overrepresented GO terms: metal ion binding and exposure to organic substances, both consistent with the view that exposure to chemical pollution has acted as a selective pressure on roach in the River Lee. We have also conducted a 2 year exposure to 1.8 ng/L ethinyloestradiol (EE2) to compare sensitivities of the offspring of roach from different rivers. Exposure resulted in a range of gonadal phenotypes in male fish; from fish with normally developed gonads to those with both male and female gametes (intersex) and an ovarian cavity. We are now comparing feminised responses between fish from different rivers.

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Integrating biotic factors in toxicokinetic modelling of metal accumulation in fish

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Fish are exposed to both pollutants and parasites. Parasites might affect the toxicity of pollutants like metals. So it is important to consider parasitism in modelling metal accumulation in fish. Toxicokinetic (one- or multi-compartment) models have been used for simulating metal accumulation in aquatic organisms. The multi-compartment model allows for predicting tissue-specific concentrations, but requires a larger number of parameters. We developed a one-compartment model and a multi-compartment model (covering blood, storage, gills, kidney, liver, intestine, and gallbladder) simulating metal accumulation in the chub *Squalius cephalus* uninfected and infected with the acanthocephalan *Pomphorhynchus laevis*. In models for the uninfected chub, uptake toxicokinetics was related to species weight and metal properties while the absorption efficiency and elimination rate constants were parameterised based on published data. For the infected chub, physiological parameters were parameterised from the value for the uninfected. Tissue-blood partitioning in the multi-compartment model was calibrated using published data on ^{210}Pb accumulation overtime, but not validated. The one-compartment model was validated using data set on Pb concentrations in the whole fish at the end of exposure experiments. Predicted concentrations of Pb in the fish-parasite system by the one-compartment model were significant related to the measured. The estimates were generally within one order of magnitude of the measurements. Moreover, the predictions of Pb accumulation in the system were more sensitive to the absorption efficiency than to the elimination rate constant. For the multi-compartment model, preliminary calibration results show high tissue-blood partitioning in liver and intestine, consistent with their role in detoxification, and effects of parasites on the tissue-blood partitioning in liver and intestine. Modelled concentrations of ^{210}Pb in all fish organs were generally within one order of magnitude of the measured. Stable concentrations in storage and increasing concentrations in intestine and gallbladder of the uninfected chub could be explained by the model. The stability in ^{210}Pb accumulation in storage and fluctuation in gills of the infected chub are consistent with modelled patterns. Yet, increasing concentrations in gills and liver of the uninfected and fluctuating concentrations in intestine and gallbladder of the infected could not be simulated by the model.

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Ecotoxicological impacts of cyanobacteria on fish: effects of chronic exposure of medaka fish to cyanobacteria-dominant conditions

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The interactive effects of eutrophication and climate change may promote cyanobacterial blooms in continental aquatic ecosystems, which pose potential risks to fish ecology and ecosystem sustainability. To date, more than 600 cyanobacterial compounds potentially toxic have been described from various taxa. These cyanotoxins can be released by cyanobacteria into the water leading to potential toxic effects to aquatic organisms. Among the various families of cyanotoxins, two classes of metabolites have attracted more attention due to their toxicity: the hepatotoxins such as microcystin (MC) and the neurotoxins. Furthermore, during the past decades, many bioactive peptides and alkaloid groups have also been discovered, as the classes of aeruginosins, microginins, cyanopeptolins and anabaenopeptins which could present also potential deleterious effects on aquatic organisms. Since cyanobacteria can produce and release mixtures of cyanotoxins rather than just single toxins, a better understanding of the production and the toxicity of this diversity of metabolites is necessary. While several works reported the toxicological effects of single cyanotoxins, only few have discussed the importance of an environmental context. A method of chronic exposure of a fish model (medaka) to cyanobacteria in an environmental relevant context was developed in this study. Female and male medaka fish were exposed by balneation during 21-days to 4 treatments: i/ Control, ii/ Non-MC producing culture, iii/ MC producing culture extracts and iv/ MC producing culture. The aim of this study was to investigate the cellular and molecular ecotoxicological effects of cyanobacterial blooms in order to better understand their potential impacts on fish, through the use of histologic examinations and "Omics" approaches in fish liver. Interestingly, our results show the immunolocalization of microcystins in the gut and the liver of microcystin-producing cyanobacteria-treated fish, but not in fish treated by other conditions. In addition, quantitative proteomics by iTRAQ 8-plex labelling and metabolomics by Nuclear Magnetic Resonance (NMR) highlighted clear metabolic differences according to gender and treatments. This work will give a first insight of effects of genuine cyanobacteria bloom on medaka fish in an environmental context and highlights the importance of taking in consideration living cells rather than extracts in fish exposition, approaching the actual conditions of aquatic systems.

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A novel ZF-Tox-Array to identify toxicity of environmental contaminants

and complex mixtures

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There are over 100,000 chemicals on the market, many of which end up in the environment as complex mixtures. The current chemical-by-chemical approach to environmental quality assessment has significant limitations, as only a limited number of chemicals can be assessed. Testing or monitoring single chemicals also does not address the complexity associated with mixtures. There is growing public concern about the risks associated with low levels of highly potent chemicals such as drugs, hormones and pharmaceuticals which end up in the environment via waste streams. Therefore, other sensitive methods are needed to assess the risk of chemicals in general and certainly when they are present in complex mixtures, like in the environment. Here, we aim to provide a new sensitive genomics-based tool for safety assessment of chemicals and complex mixtures. Therefore we developed ZF-Tox-Array, a combination of state of the art multiplex qPCR and zebrafish (*Danio rerio*) toxicity testing, as a new cost-effective, sensitive effect-based approach to screen chemicals and environmental samples for their toxicity. With the ZF-Tox-Array, multiple toxic modes of action (MoA) can be assessed in parallel compared to standard applied *in vitro* assays which are mostly specific for only one MoA. Our new system allows to screen up to 42 target genes, covering several toxicity pathways in parallel. Based on literature, target genes were selected known to be involved in commonly assessed toxicity pathways was made. MoA covered general stress, apoptosis, oxidative stress, DNA damage, metabolism, metabolism phase II/III, endocrine disruption, adipogenesis, insulin signaling and neurotoxicity. A great advantage of our ZF-Tox-Array system is the flexibility as the list of target genes can be adapted to a variety of MoA of interest. The ZF-Tox-Array system was first validated and optimized using model substances. In the next step we applied our new system to screen 39 environmental pollutants for their neurotoxic potential using a special set of neurotox target genes. In an additional study we selected 42 target genes covering different MoAs that are commonly studied with water risk assessment and screened different drinking, surface and waste water samples for their toxicity. Our study shows that even at concentrations where no visual malformation can be seen strong gene expression changes can be observed and that new toxic insights can be gained by using ZF-Tox-arrays.

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Stickleback FET test with gene expression analysis can detect endocrine disrupting chemicals

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The three-spined stickleback (*Gasterosteus aculeatus*) is a useful sentinel species in chemical risk assessment, particularly for detecting endocrine disruptors (EDs). As with zebrafish, the fish embryo toxicity (FET) test using stickleback needs to be developed as an alternative to adult fish test in compliance with animal welfare. Gene expression analysis following FET test can provide an insight on the signalling pathway responding to chemicals and as such can provide an early warning for downstream effects. To understand the differences in transcriptional response to chemicals between adult fish and embryos, we performed global gene expression analysis of the embryos using microarrays (Agilent-029767) following FET test. In the FET test, we exposed fertilized stickleback eggs to the 6 single chemicals (cadmium, dibutyl phthalate, fluoxetine, ibuprofen, 17α-ethynylestradiol(EE_2) and levonorgestrel) and 6 mixtures, each consisting of 5 chemicals for 10 days (the time needed for the stickleback embryo to reach the self-feeding stage). The microarray results were compared with those obtained from adult fish liver after 4 days of exposure. Our overall aim was to investigate if the fish embryos were capable of expressing at least partly the signalling pathway from molecular initiating event to adverse outcome and evaluate the potential replacement of adult fish by embryos. No. of genes statistically significantly expressed in 6 single chemicals from controls was as follows: $EE_2 = 3$, Levonorgestrel = 10, DBP = 875, Cd = 940, Ibuprofen = 1589, Fluoxetine = 0. Similar response was found after Cd, ibuprofen, and DBP exposure, suggesting typical chemical stress response (e.g. oxidative stress). For EE_2, estrogen related genes (vitellogenin, choriogenin) were upregulated as was previously found in adult fish. For levonorgestrel, the androgen related genes (spiggins) were upregulated, which was not observed in the liver of adult fish (spiggin is expressed only in the kidney of adult fish). Levonorgestrel antagonised the action of EE_2 by significantly reducing the expression of vitellogenins and choriogenins, whilst EE_2 antagionised the action of levonorgestrel by reducing the expression levels of spiggins. Both findings were identical to those observed using adult fish. These results indicate that the stickleback FET test is a useful screening assay for EDs, providing clear responses to both estrogenic and androgenic chemicals.

MetaOMICs in ecotoxicology: evaluation of alterations in the structure and functions of ecosystems

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Next-Generation Sequencing to highlight community changes in river biofilms linked to pharmaceutical loads from a wastewater treatment plant

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Hospital wastewaters contain wider spectrum and higher concentrations of pharmaceuticals than urban ones. Despite this, they are usually co-treated in traditional wastewater treatment plants (WWTP) which are not designed to remove specific hospital contaminants. Hence urban and hospital treated effluents may still contain residues of pharmaceuticals which are then released into the recipient aquatic environment and might cause environmental risk. Therefore, a better understanding of the effect of pharmaceuticals in the environment is required. Biofilms are effective “biological sensors” for assessing the environmental effects of pharmaceuticals due to their ability to respond rapidly to physical, chemical and biological fluctuations by changes in their structure and composition. The use of Next-Generation Sequencing (NGS) allows a deeper and more accurate characterization of changes in the community structure. This is helpful to describe specific adaptations of bacterial and algal communities to the presence of pharmaceuticals and thereby disentangle multiple stress effects in environmental studies. Environmental biofilms were colonized (6 times from Feb to July 2014) at the output of a wastewater treatment plant 1/ in separately treated effluents from urban and hospital wastewaters and 2/ in the recipient river (River Arve, French Alps) up- and downstream from the WWTP output. A joined monitoring of environmental abiotic factors (SIPIBEL observatory) and pharmaceuticals (POCIS) was performed. NGS, using PGM Ion Torrent technology, was used for in-depth characterization of the composition of diatoms and bacterial communities in the biofilms through a metabarcoding approach based on rbcL and 16S rRNA amplicons, respectively. Concentrations of pharmaceuticals were higher in hospital than in urban effluents. Lower concentrations were observed in the river than in effluents, although higher downstream than upstream the WWTP output. NGS sequencing showed strong differences between microbial biofilm communities developed in all 4 sites, together with a clear seasonal effect. However local similarities were stronger than seasonal ones, revealing the strong impact of the wastewater effluents in shaping the environmental communities. Finally, the assessment of community composition using NGS, coupled to integrated measurement of pharmaceuticals, is efficient to highlight the disturbing effect of pharmaceuticals on environmental microbial communities.

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Structure of microbial communities impacted by hydrocarbons in oxic/anoxic interface in coastal marine sediments

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Coastal marine sediments constitute unique functional ecosystems constantly subjected to oxic/anoxic oscillations according to tide cycles, bioturbation activities by macrofauna and other mechanical processes. Due to their location they are exposed to pollution injuries such as oil spills. Microbial communities play critical roles in global biogeochemical cycles in marine sediments, particularly in response to the presence of hydrocarbons. Thus, understanding the response of these key microorganisms exposed to hydrocarbons under oxic/anoxic oscillations provide pivotal information for estimating the resilience capacities of the ecosystem. Recently, we showed that the organization of hydrocarbon degrading bacterial communities was driven by sediment reworking activities (Stauffert *et al*., 2013). Indeed, three different microbial communities were obtained applying various reworking strategies but their overall degradation efficiency was similar highlighting the functional redundancy involved in hydrocarbons degradation. In order to further characterize the microbial assemblages, the three microbial communities were incubated in different oxygenation regimes (oxic, anoxic and oscillating conditions) in bioreactors. Then *Bacteria* and *Archaea* 16S rRNA transcript amplicons were analysed by high-throughput sequencing. Although PICRUSt functional analyzes (Langille *et al*., 2013) revealed that the three *Archaea* communities were different, they were not affected by the oxygenation conditions imposed in the bioreactors. In contrast, *Bacteria* communities were structured according to the oxygenation conditions. Correlation analysis showed that *Archaea*/*Bacteria* interactions, particularly the methanogens/sulfate-reducing bacteria relationships, are of primarily importance in the microbial communities assemblages in presence of hydrocarbons including the hydrocarbon degradation capacity. We thus suggest to carefully examine the *Archaea*/*Bacteria* interactions in microbial ecotoxicology studies to evaluate the impact of pollutants. Langille MGI, Zaneveld J, Caporaso JG, McDonald D, Knights D, Reyes JA, *et al*. (2013). Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. *Nat Biotechnol* **31**: 814–821. Stauffert M, Cravo-Laureau C, Jézéquel R, Barantal S, Cuny P, Gilbert F, *et al*. (2013). Impact of Oil on Bacterial Community Structure in Bioturbated Sediments. *PLoS ONE* **8**: e65347.

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Large-scale shotgun metagenomics of periphyton communities exposed to triclosan

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High-throughput DNA sequencing has a great potential to detect effects from toxicants in natural communities. However, its potential in community ecotoxicology has so far not been realized. In this study we test if high-throughput Illumina shotgun sequencing can identify effects of the bactericide triclosan (TCS) in natural marine periphyton (biofilm) communities. We performed a long-term (17 days) flow-through microcosm experiment with natural marine periphyton. These communities are highly diverse and a large sequencing effort is needed to achieve a good sequencing depth. 4 replicate controls and 8 TCS exposure levels, covering a concentration range of 0.32-1000 nM with and triplicates of the 3.16 nM, 31.6 nM and 316 nM treatments, was included.Paired-end sequencing libraries was prepared with the TruSeq PCR-free kit and sequencing was done using the HiSeq 2500 Illumina sequencing platform. We generated 475 Gbases (1.9 billion DNA read pairs) of metagenomic sequence data, which after quality control summed up to ~ 3,5 billion sequences. After assembly we obtained 1,284,307 scaffolds with an average N50 value among the samples of 3662 bp. The longest scaffold was 596,794 bp, which corresponds to half a genome of a small genome-sized free-living prokaryote. In addition, we generated 313,855 16S and 176,566 18S amplicon sequences to further explore the effects of TCS on community structure, and to compare shotgun and amplicon sequencing approaches. Bioinformatic analyses are ongoing, but first annotation results using TIGRFAM identified genes from 3415 functional classes, of which 656 were differentially abundant in unexposed control communities vs. communities exposed to 316 nM TCS. The composition of these protein-encoding gene families in control communities and communities exposed to 316 nM TCS was cleary different. TIGRFAMS that were found to be significantly more abundant in exposed communities included, for example, those for protein and energy metabolism, metal resistance and various transporters (including ABC-transporters). TIGRFAMs significantly more abundant in unexposed controls included, for example, many genes encoding photosynthesis proteins. We will also present further analyses of TCS effects on functional and taxonomic composition, as well as analyses of proteins correlated to TCS tolerance. To our knowledge, this is the first large-scale metagenomics study of toxicant effects on microbial commuities.

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Characterization of environmental metallothioneins: a new metallothionein family isolated from soil eukaryotic metatranscriptome.

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Human activities can cause soil metal pollutions and therefore modify soil ecosystem functioning. Eucaryotic microorganisms are an important element in ecosystems: for example, the eukaryotic predators regulate bacterial populations by grazing them. If a metal pollution occurrs, organisms have to provide resistance mechanisms to prevent toxic effects on cell components, like DNA damage or metabolism disturbance. For this purpose, intracellular chelation is an important mechanism enabling organisms to limit metal toxic effects by synthesis of metal binding molecules with a high cysteine content such as metallothioneins (MTs). MTs are a superfamily of low molecular weight proteins rich in Cys (15-33%), divided into 15 different families depending on their amino acid sequence features. They have a physiological dual role: metal homeostasis in physiological conditions and protector in response to metal excess. Here, by a functional metatranscriptomic approach, we isolated for the first time eukaryotic environmental metallothioneins (EMTs). Their DNA sequences were recovered by specific PCR in diverse soil samples revealing that the organism(s) bearing these genes is(are) ubiquitous. We have characterized EMTs with respect to their amino acid sequence features and to their metal binding abilities (studied by ESI-TOF-MS after protein overexpression in *E.coli* in the presence of Zn, Cd or Cu and subsequent purification of the respective metal complexes). EMT sequences present particular properties that strongly relate them to typical MTs: a high content of conserved Cys residues (20-24%), Cys residues mainly distributed in specific sequential motives (1 CCC, 2 CXCC, 2-3 XCCX, and 4 CXC), and no aromatic amino acids. But they have unusual features too: an “important” length (110-132), the presence of CCC clusters, two conserved His residues and a cysteine-free sequence linker dividing the protein sequence in two parts. After purification of the corresponding metal complexes, ESI-TOF-MS analysis revealed that EMT proteins are able to bind Cd, Zn and Cu, likewise the MT proteins. In most of the cases : i) some mixtures of metal-protein species, ii) the presence of sulphide ligands in the Cd-EMT complexes, and the presence of heteronuclear Zn,Cu-EMT species, as the result of the Cu-supplemented syntheses, were observed suggesting that EMTs have no specific metal binding preferences. These data reveal that EMTs define a new metallothionein family.

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DYMICO, metaproteomic analysis of environmental microbiome dynamics as response to pollutants and stressors

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Metaproteomic analysis could provide a functional overview from several level of complexity: at the community level, how the communities respond to the exposure or stress; at the molecular level, which protein families have been modified their level of expression; and at the biochemical level, which catalytic reaction with relevant role to maintain the community in equilibrium. We presented the metaproteomic analysis of aquatic sediments from Baltic Sea exposure to propranolol and salinity changes for 6 weeks. We tested if a metaproteomics-based assessment can estimate: i) variation in the abundance of protein families, ii) changes in biodiversity, and iii) what are the key players in the community that drive the variation in the community and the adaptation to the exposure to pollution and to abiotic changes in the environment. The SDS-boil method for protein extraction has increased the amount of protein recovered as well as the biodiversity. This method was offering the highest values for reproducibility among biological replicates. Our results indicate that two step in the data analysis pipeline are crucial to implement this methodology for environmental assessment: the selection of the database or the utilization of an in-house database for protein identification, and the comparison between search engines. We compare our results from two database: Cameraand NCBI non-redundant repository database and the LC-MS (2)-spectra were searched using a combination of two different search engines: Unipept or MEGAN against different database. The salinity stressor has an important impact into the community response. Our results indicated the analysis of microbial community dynamics could be correlated to both changes in the molecular responses of the communities, interspecies interaction and therefore interprete and predict the ecosystem response to stressors or exposure to pollutants. Furthermore, we can explore the prediction capabilities of these methodologies to develop a metaproteomics-based environmental assessment.

Pollutant risks to amphibians and reptiles: how much we know and what we need

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Compared ecotoxicity of raw and oxidised carbon-based nanoparticles on Xenopus laevis larvae

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Carbon-based nanoparticles (CNPs) such as carbon nanotubes or graphene have numerous attractive properties leaching to their use in many application fields whether they are raw or functionalised, such as oxidised. More and more produced, these nanoparticles may finally contaminate the aquatic environment, which is a major receptacle of pollutants. This work consists in assessing the ecotoxicological risks induced by a range (from 0.05 to 50 mg.L-1) of double-walled carbon nanotubes (DWCNTs) and few layer graphene (FLG) on the amphibian *Xenopus laevis*. We studied the influence of surface chemistry by comparing raw materials with their functionalised counterparts by testing different oxidation levels and thus different nanoparticles: oxidised DWCNTs

(DWCNTs-ox), graphene oxide (GO), and reduced graphene oxide containing respectively 4 and 17 % (atomic composition) of oxygen (rGO 4%, rGO 17%). Tests have been carried out with a standardised protocole (ISO, 2006). We focused on various endpoints: (i) mortality, (ii) growth inhibition and (iii) genotoxicity (induction of micronucleated erythrocytes). The mortality was not significantly impacted whatever the type of nanoparticle and tested concentration. In contrast, a significant growth inhibition was observed from 10 $mg.L^{-1}$ of raw DWCNTs and FLG, and at 50 $mg.L^{-1}$ of DWCNTs-ox and GO. On the contrary, growth significantly increased for larvae exposed to 1 $mg.L^{-1}$ of rGO 4% and rGO 17% comparatively to the negative control. No genotoxicity was noted for the raw nanomaterials nor for rGO 4 and 17 %, but significant genotoxicity was observed for DWCNTs-ox at 1 $mg.L^{-1}$ and for GO at 0.1 $mg.L^{-1}$. The chronic toxicity observed appeared to be limited to physical effects (intestinal obstruction and/or abrasive effects and/or nutrients deprivation and/or gill clogging). However, genotoxic effect could be attributed to intrinsic properties of oxidised nanoparticles. This work has been carried out in the framework of the Graphene Flagship program. Key words: carbon-based nanoparticules, oxidation, ecotoxicity, *Xenopus laevis* References ISO 21427-1. International Standard. Water quality - Evaluation of genotoxicity by measurement of the induction of micronuclei - Part 1: Evaluation of genotoxicity using amphibian larvae. ISO 21427-1, ICS: 13.060.70, Genova – CH (2006). Presentation preference: platform presentation

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Effects of a glyphosate-based herbicide and temperature on the development of Common toads (Bufo bufo, L.; Amphibia: Anura)

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Herbicides based on the active ingredient glyphosate are increasingly applied in agriculture, horticulture and private gardens all over the world. Recently, leaching of glyphosate or its metabolite into water bodies inhabited by amphibians has been reported. However, very little is known about non-target effects of these herbicides on amphibians and even less is known to what extent different temperatures might alter these effects. Using climate chambers, we investigated the effects of the glyphosate-based herbicide Roundup PowerFlex® (480 g L^{-1} glyphosate, formulated as 588 g L^{-1} potassium salt) on the larval development of Common toads (*Bufo bufo*, L.; Amphibia: Anura) under different temperature regimes (15°C vs. 20°C). We established five herbicide concentrations: 0, 1.5, 3, 4 mg acid equivalent L^{-1} and a 4 mg a.e. L^{-1} pulse treatment (totally three applications of 1.5, 1.5 and another 1 mg a.e. $L^{-}1$) at each temperature in a full-factorial design. Each treatment combination was replicated 5 times; the experiment ran for 24 days. Results showed no effect of herbicide concentration on body length and body width but a highly significant effect of temperature on these growth parameters. Moreover, highly significant interactions between herbicide and temperature on body length and body width were observed suggesting that herbicides had different effects on different temperatures. \n In conclusion, although Roundup PowerFlex® at the tested concentrations appeared to have no acute toxicity to larvae of Common toads, the observed effects on tadpole growth will potentially affect competitive interactions in spawning ponds of amphibians. Our findings of herbicide x temperature interactions might become more prevalent when human-induced climate change will lead to more extreme temperatures.

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Standardize or diversify conditions in experimental ecotoxicology? A case study on herbicide toxicity to larvae of two anuran amphibians

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As part of the biodiversity crisis, amphibian species are experiencing population declines and extinctions throughout the world. One of the major causes of these declines is increasing pesticide use. Even though there is a steeply increasing number of ecotoxicological studies treating the effects of pesticides on non-target organisms, studies assessing the adequacy of different experimental approaches have remained scarce. We scrutinized effects of a glyphosate-based herbicide on larvae of two European anuran amphibians by estimating species-specific LC50 values. To assess how an additional factor may influence outcomes of a standard toxicity test, we investigated whether predation threat exacerbated the effects of the pesticide. Finally, to study how repeatable the measured effects are across experiments, we performed experiments repeatedly. We exposed agile frog (Rana dalmatina) and common toad (Bufo bufo) tadpoles to two predator treatments (no predator, dragonfly larvae) combined with seven (0, 0.009, 0.03, 0.24, 1, 2, 6.5 mg a.e. / L glyphosate) and nine (0, 1, 2, 3, 4, 5, 6, 7, 8 mg a.e. / L glyphosate) herbicide concentrations in a full factorial design and repeated the experiment with a subset of the experimental treatments and partly with slight modifications one week later on another set of animals. Our results indicated that the herbicide was moderately toxic to the studied animals. The presence of chemical cues on predation threat increased the toxicity of the herbicide in case of agile frog tadpoles, but, surprisingly, made the herbicide less lethal for common toad tadpoles. The repeatability of the experimental results across experiments differed between the two species: the estimated sensitivity of agile frog tadpoles varied considerably, while in case of the common toad LC50 values remained very similar across experiments. Our results support the hypothesis that the effects of pesticides can be significantly influenced by additional stress factors and by slight changes in the experimental setup, all also depending on the studied species. This draws attention to the suggestion that strict standardization may not deliver widely applicable insights into the toxicity of contaminants and, instead, introducing some variation into the design of future ecotoxicological experiments may prove highly beneficial.

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New histological and molecular biomarkers for developmental reproductive toxicity in Xenopus tropicalis

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The risk posed by plant protection chemicals to amphibians shall be evaluated according to the EU Plant Protection Product Regulation. To accomplish this, amphibian test methods for investigating adverse effects of chemicals need to be developed. The present study aimed to develop the *Xenopus tropicalis* test system for developmental reproductive toxicitybycharacterising molecular and histological features of sexual development**.** The ontogenetic development of the Müllerian ducts (precursors of the female reproductive tract) was characterized histologically. In addition, the mRNA expression of *amh* (*anti-Müllerian hormone*), *amhr2* (*amh receptor 2*),*intracellular and membrane progesterone receptors* (*ipgr* and *mpgr beta*) and *cytochrome P450 19a1* (*cyp19a1)* were determined in the urogenital complex (composed of kidney tissue, gonads and sex ducts). *amh* expression was evaluated as a molecular biomarker for phenotypic sex.The animals were sampled for mRNA analysis duringsex differentiation at Nieuwkoop and Faber (NF) stage 51 and 56, and at 4 weeks post-metamorphosis. Gonadal and Müllerian duct development were characterized histologicallyat 4 weeks post-metamorphosis. As *X. tropicalis* displays a high variability in larval developmental rate, the sampling scheme was designed to obtain both age- and stage matched groups.The results show that the *amh* mRNA expression levels were higher in individuals with low *cyp19a1* expression (ovarian marker) and vice versa. The sexually dimorphic expression profile was more distinct for *amh* than for *cyp19a1*, supporting our hypothesis that *amh* expression is useful as a testicular biomarker during gonadal differentiation in *X. tropicalis*.The *pgrs* expression levels increased over the studied period and showed no sex differences. The*amhr2* expression level was higher in females than in males at NF 56 and at 4 weeks post-metamorphosis. The histological evaluation showed that folliculogenesis had initiated and that the Müllerian ducts were larger in females than in males. The proportion of follicular oocytes in the ovary at 4 weeks post-metamorphosis increased with increasing time for the individual to complete metamorphosis, emphasizing the importance of having study groups that are both age- and stage matched in toxicity studies of larval amphibians. This new knowledge on sexual development in *X. tropicalis* is useful in the development of early life-stage endpoints for developmental reproductive toxicity.

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Acute oral toxicity in terrestrial life stages of amphibians: comparisons to birds and mammals

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Amphibians are currently the most threatened and rapidly declining group of vertebrates and this has raised concerns about their potential sensitivity and exposure to plant protection products and other chemicals. Current environmental risk assessment procedures rely on surrogate species (e.g. fish and birds) to cover the risk to aquatic and terrestrial life stages of amphibians, respectively. Whilst a recent meta-analysis has shown that in most cases amphibian aquatic life stages are less sensitive to chemicals than fish, little research has been conducted on the comparative sensitivity of terrestrial amphibian life stages. Therefore, in this paper we address the questions “What is the relative sensitivity of terrestrialamphibian life stages to acute chemical oral exposure when compared with mammals and birds?” and “Are there correlations between oral toxicity data for amphibians and data for mammals or birds?”. Identifying a relation between these data may help to avoid additional vertebrate testing. Acute lethal oral amphibian toxicity data collected from the scientific literature and ecotoxicological databases were compared with toxicity data for mammals and birds. It was found that toxicity data for terrestrial amphibian life stages are generally sparse, as noted in previous reviews. However, the single-dose oral toxicity data for terrestrial amphibian life stages that are available for 23 chemicals suggest that oral toxicity to terrestrial amphibian life stages is similar to or lower than that for mammals and birds, with a few exceptions. Thus, mammals or birds are considered adequate toxicity surrogates for use in the assessment of the oral exposure route in amphibians.

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Do developmental stage and route of exposure determine pesticide effects on lizards?

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Reptiles are an understudied group in ecotoxicology and the risk of pesticides on them is supposed to be covered by bird and mammal toxicity data derived from oral exposures. However, topic exposure during egg incubation, as well as dermal uptake and/or overspray of pesticides once hatchlings leave the nest could constitute relevant exposure ways for reptiles. We conducted a two-stage exposure of wall lizard (*Podarcis muralis*) eggs and hatchlings to three pesticides in order to analyse the effects of pesticide exposure on each developmental stage and to compare the relative importance of dermal and oral exposure of juveniles through different exposure routes. Eggs were incubated in soils previously sprayed with a pesticide solution corresponding to 40% of the field application rate (to consider relevant crop interception) of formulations of either glufosinate ammonium (herbicide), folpet (fungicide) or fenpyroximate (insecticide). Eggs exposed to folpet hatched earlier and with a lower body mass than controls, whereas fenpyroximate reduced body size at hatching. Upon hacthing, animals were assigned to a juvenile treatment consisting in the exposure through spiked soil (dermal) or food (oral) in a factorial block design considering both in-ovo and hatchling exposure. Concentrations during the hacthling exposure corresponded to 25% of the application rate. Interestingly, egg exposure but not hatching exposure determined the body mass gain during the post-hatch stage of lizards exposed to glufosinate ammonium or fenpyroximate. Oxidative stress biomarkers showed different trends as a function of life stage; the activity of glutathione peroxidase was increased after egg exposure to folpet and glufosinate ammonium and also after juvenile exposure to any pesticide. However, in animals exposed to folpet as eggs, further exposure to the fungicide as juveniles reduced the activity of the enzyme, which could be interpreted as a hormetic response consequence of a saturation of the enzyme synthesis under prolonged exposures to folpet. Effects of pesticides on juveniles exposed either dermally or orally were very similar, which suggests the conenience of considering dermal exposure in risk assessment for reptiles as an additional route of pesticide uptake. In addition, our result highlight the importance of considering lagged effects in pesticide risk assessment. Financed by the EU's FP7 (IEF-2012-328328)

Are we going about chemicals risk assessment for the aquatic environment the wrong way?

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Assessing risks and regulating the many 1000s of man-made chemicals how can we cope?

J.P. Sumpter, Brunel University / Institute of Environment Health and Societies

Tens of thousands of man-made chemicals are in everyday use in developed countries. A high proportion of these, or their transformation products, probably reach the aquatic environment. A considerable amount is known about the environmental concentrations of some of these chemicals (such as metals), especially the regulated ones, but little or nothing is known about the majority. In densely populated countries, most or all rivers will receive both diffuse (e.g. agricultural runoff) and point source (e.g. sewage treatment plant effluent) inputs, and hence be contaminated with complex, ill-defined mixtures of chemicals. Most freshwater organisms will be exposed, to varying degrees, to this contamination. The number of species exposed is in the thousands, and quite possibly tens of thousands. Little is known about whether or not these species are adversely affected by the chemicals present in their environment. Often it is not even known what species are present, let alone whether they are affected by the chemicals present. In a few high-profile cases (e.g. tributyl tin causing imposex in molluscs and oestrogens 'feminizing' male fish), chemicals have undoubtedly adversely affected aquatic species, occasionally leading to population crashes. Whether or not other chemicals are affecting less visible species (such as most invertebrates) is largely unknown. It is possible that only very few chemicals in the freshwater environment are adversely affecting wildlife, but it is equally possible that some effects of chemicals are, as yet, undiscovered (and may remain so). Nor it is clear which chemicals may pose the greatest risk to aquatic organisms. All these uncertainties leave much to chance, yet designing a regulatory system that would better protect aquatic organisms from chemicals is difficult. A more flexible and intelligent strategy may improve the current situation. Finally, the risk due to chemicals is put into context with the many other threats, such as alien species and new diseases that undoubtedly can pose significant risks to aquatic ecosystems.

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The prioritisation of substances and the derivation of Environmental Quality Standards under the Water Framework Directive - how did we get to where we are?

D. Leverett, WCA-Environment Ltd

The WFD aims to provide a holistic approach to managing the water environment in Europe, and brings together objectives to protect the water environment from the effects of chemical pollution and broader ecological objectives, designed to protect the structure and function of aquatic ecosystems themselves. Under the WFD, the overall environmental status of a waterbody (be it river, lake, estuary or coastal) is determined by the assessment of its ecological and chemical status. Chemical status is based on the comparison of the measured concentrations of 'Priority Substances' with their substance-specific Environmental Quality Standard (EQS), which represents the concentration below which effects on populations of organisms are not expected to occur. The list of 'Priority Substances' regulated under the WFD is updated on a 4-5 year cycle, and currently comprises around 40 substances or groups of substances. While this set of substances is considered (at least under the auspices of the WFD) to represent those with the potential to cause the greatest harm to the environment, it clearly does not comprise all substances with demonstrated adverse effects on aquatic organisms nor even all such substances that have been detected as being present in European waters. How then have we ended up with this particular set of substances? The first part of this presentation will therefore outline and review the approaches and procedures that have been, and are currently, used to identify and prioritise candidate substances for potential regulation under the WFD. Once candidate substances have been identified and prioritised as candidates, the next stage in the review process is to compile and evaluate as much existing information on each candidate substance as possible, in order to undertake a preliminary risk assessment. At this stage, all data on the potential effects of a substance are subject to in-depth scrutiny and this usually generates much debate (and often disagreement) amongst those responsible for deriving the concentration deemed 'safe' for the environment. This debate generally focuses on the quantity and quality of the underlying ecotoxicity studies, and specifically the reliability and relevant of certain studies (usually those that have generated effect thresholds at the lowest concentrations, and on the magnitude of assessment factors applied to such thresholds in order to account for the uncertainties inherent in the assessment. The second part of this presentation will therefore deal with the techniques used to derive Predicted No Effect Values (PNECs; the precursors to EQS), the types of ecotoxicological data considered relevant in predicting population-level effects, the quantity of data required, the approaches used to assess the reliability of the underlying data, the uncertainties in the process, and the use of assessment or safety factors in deriving EQS. In this part of the presentation, we will also address the criteria for the assessment of persistent, bioaccumulative and toxic substances (PBT), and how the outcomes of the PBT assessment affect the future regulation of substances. While considerable scrutiny is placed on such effects data, the data on measured concentrations in the environment generally receives much less focussed attention (both at the prioritisation and preliminary risk assessment stages). Exposure data is often derived from only a very small, and largely unrepresentative, series of monitoring points in European waters, and is generally not subject to the type of in-depth reliability and relevance assessments applied to effects data. Nevertheless, such data is critical in the prioritisation process, and a lack of quantity and/or quality in exposure data has at least an equal effect (and debatably more so) on the outcomes of the process as the ecotoxicological effects information. Therefore, the final part of this presentation will cover the identification and evaluation of exposure data, and measures to improve this element of the overall assessment, including the WFD Watch List.

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Does the current risk assessment of chemicals underestimate toxic stress in Europe?

P. Von der Ohe, UBA - Federal Environment Agency / IV Pharmaceuticals

There is evidence that anthropogenic chemicals can have profound local and regional effects on aquatic communities, while the overall relevance of chemicals regarding larger spatial scales remains mostly unknown. The reason for this are both the lack of comprehensive monitoring datasets as well as the availability of respective ecotox-data. Here we present the first risk assessment of organic chemicals on a continental scale. The study is based on regulatory monitoring data for a total of 4,000 monitoring sites available from the European Environmental Agency for the years 2008-2012. For the 223 organic substances monitored, the available experimental ecotox-data was collated and supplemented by QSAR predictions in case of data gaps. Results showed that organic chemicals are likely to exert acute lethal and chronic long-term effects on sensitive fish, invertebrate, or algae species in 14 % and 42 % of the sites, respectively. Pesticides, brominated flame retardants, and tributyltin were the major contributors to the overall chemical risk. Their presence was related to agricultural and urban areas in the upstream catchment. Moreover, the risk of potential acute lethal and chronic long-term effects increased with the number of eco-toxicologically relevant chemicals that have been analyzed at each site. However, as most monitoring programs considered in this study often included only a subset of these chemicals, our assessment is likely to underestimate the actual risk. \n Finally, we analyzed whether the observed chemical concentrations had any effects on aquatic communities. For that reason, we used a sub-set of sites in France where many of these relevant chemicals have been analyzed and concurrent community data was available. We found that an increasing chemical risk was associated with increased deterioration in water quality indices for fish and invertebrates communities. Our results therefore clearly indicate that chemical pollution is a large-scale environmental problem that requires far-reaching, holistic mitigation measures to preserve and restore ecosystem health. It also suggests that current risk assessment approaches on a substance by substance bases are likely not protective for the environment. This is especially so when considering that the large and chemically diverse group of pharmaceuticals has not been included in this study, due to a lack of monitoring data as well as appropriate ecotox data that considers the specific effects of these substances in wildlife. Examples for the latter include the feminization of the male roach (*Rutilus rutilus*), a widespread group-spawning fish in English rivers, due to water-borne estrogens, or the population decline of the oriental white-backed vulture (OWBV; *Gyps bengalensis*), which was associated with renal failure and visceral gout after feeding on prey treated with anti-inflammatory drugs.

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Forwarding sustainable use of chemicals via ecological impact analyses: the eco-epidemiological approach
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Monitoring data have delivered us only fragmented insights in chemical exposures of biota (man and species in ecosystems) and – in fact – very little insights in net impacts. With approx. 80-100k chemicals produced regularly by man, exposing millions of species, the question is whether we are on the right track in the scientific enterprises underpinning chemical safety regulations. The tome '*Chemicals without harm'* (Geiser, 2015, reviewed in *Science*, DOI 10.1126/science.aac9931) collates some visionary approaches towards a safe and sustainable chemical economy. Prioritizing the design of novel chemicals and products being a key step. But logically, we also need to fill the gap between the low percentage of chemicals being considered by regulations and the plethora of chemicals and mixtures out there. Eco-epidemiological analyses have been proposed already in 1984 to diagnose impacts of chemicals and their mixtures in ecosystems. It is only in the last decade that this diagnostic approach has gained further attention, enabled by the large growth of monitoring data as well as analytical modelling power. This contribution describes the eco-epidemiological approach and how preventive regulatory action on various chemical groups together with field impact assessments have brought the field forward. The approach starts by ranking sites where ecological impacts are highest and where this is likely to be attributable to chemical mixtures, then in ranking contributions of specific chemical(s) (groups) in those effects. This enables a solution-focused approach to abatement to be chosen. Examples are presented from a decade of eco-epidemiological diagnostic analyses, in the context of solution-focused approach to forward the sustainable use of chemicals.

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Does evidence from field population studies of aquatic wildlife suggest laboratory ecotoxicology information is misleading?
A. Johnson, CEH Wallingford / Wallingford
With endocrine disruption the evidence from aquarium tests together with the inspection of wild fish anatomy pointed to a threat to fish populations living in proximity to sewage effluents. This issue has in part led the EU for the first time to put three 'pharmaceuticals', ethinylestradiol, estradiol and diclofenac, on a watch list potentially leading to them being priority substances needing control. Despite these concerns and enthusiasm for control, we still don't know if fish populations are actually being harmed by pharmaceuticals or estrogens in sewage (wastewater) effluent. It is important to distinguish here between harm to individuals (for which evidence certainly exists for estrogens) and harm to populations. Harm to individuals is a matter of regret, whilst harm to populations is a potential catastrophe. Recent evidence implies that we may not be facing a disaster. Compilation of fish catch data caught for recreation in the UK has been on an upward trend since the 1970s. We have recently compared the abundance of wild fish populations in the UK with wastewater exposure and over the past 10 years and there was no relationship. Looking at annual trends in abundance of different fish species has not shown declines in response to years of high wastewater exposure. In some cases roach (Rutilis rutilis) populations have grown in response to years of higher effluent exposure. Similarly, macroinvertebrate families do not seem to behave as we expect. Thus, whilst diversity is reduced with wastewater exposure, the trends in individual families are not so predictable. Thus, population abundance can grow in years were high wastewater exposure occured. The prevalence of sensitive families was not linked to levels of wastewater exposure. Nevertheless, some would argue that the precautionary principle requires us to act where a concern exists, even though the evidence may be insubstantial. However, governments still require some calculation of proportionality to be carried out. In other words, are the costs of a remedy proportional to the threat? This puts regulators in a dilemma, since the key evidence of harm to fish populations is lacking. Scientists are not the decision makers and regulators may still proceed on the evidence of harm to individual wildlife. But evidence on the state of populations in response to the challenge of chemicals in effluent still remains a great unknown in the debate.

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Discussion
A. Johnson, CEH Wallingford / Wallingford

Consensus building in life cycle impact assessment: experiences, achievements and challenges

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General description and introduction
R. Frischknecht, treeze Ltd.
Improving life cycle impact assessment models to be applied in the integrated environmental assessment of products is crucial. Several efforts are ongoing to address this need of improvement. To answer this, the UNEP---SETAC Life Cycle Initiative (2012---2017) has launched a flagship project to provide global guidance and build consensus on environmental LCIA indicators (see http://www.lifecycleinitiative.org/). The flagship project is focusing on building Improving life cycle impact assessment models to be applied in the integrated environmental assessment of products is crucial. Several efforts are ongoing to address this need of improvement. To answer this, the UNEP-SETAC Life Cycle Initiative (2012–2017) has launched a flagship project to provide global guidance and build consensus on environmental LCIA indicators (see http://www.lifecycleinitiative.org/). The flagship project is focusing on building consensus for different impact categories. A Pellston workshop (to be held in January 2016) will be devoted to find consensus on a first set of impact categories, namely: land use impact on biodiversity, water depletion, respiratory inorganics, climate change. The overall LCIA framework as well as several cross cutting issues will be also discussed. A common case study is being developed and serves to test and evaluate the harmonised impact indicators and to ensure their practicality. The aim of the special session is to report the result of the Pellston workshop and to discuss the way forward, e.g. in policy and business related contexts. Regarding the European policy context, the European Commission is aiming at a similar process covering land use related impact at midpoint, water related impact, respiratory inorganics and resource depletion to be used in the context of the Product Environmental Footprint (PEF) studies. Progress on this impact assessment harmonization process will also be presented in this session. Acceptability and applicability of the methods will be discussed aiming at improving the use of the methods in practice and the interpretation of the results. The session will also place some emphasis on the balance between continued methodological development and the need for stability and consensus for more efficient use of life cycle approaches in policy and private sector, supporting decision-making and communication.

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The WULCA consensus for water scarcity footprints: Assessing impacts of water consumption based on human and ecosystem demands
A. Boulay, CIRAIG - École Polytechnique de Montréal / Chemical engineering department
The need for consensus-developed and recommended methods for water use impact assessment is clear in order to perform a water scarcity footprint consistently with ISO 14046:2014 and for consistently assessing water consumption impacts in LCA. This challenge was undertaken by the WULCA working group, of the UNEP-SETAC Life Cycle Initiative in 2013. Including method developers and experts from different fields, the group developed a consensus-based indicator to assess impacts from water consumption at the midpoint level, complying with the requirements of the ISO document. This work presents the recommended methodology for performing a water scarcity footprint including a case study application. The process started with the identification of the question to answer and three proposals which emerged from the three expert workshops held in Zurich, San Francisco and Tsukuba in 2014. The group then selected criteria used to evaluate the proposals which led to a preliminary recommendation presented in 2015. From this recommendation and the testing phase that followed, additional specific and influential modeling choices were identified, analyzed and adjusted accordingly, and sensitivity analysis were performed on the most uncertain aspects. These choices and analysis are presented, which include the span of the indicator, the different choices of spatio-temporal aggregation and their meaning when the native resolution of the indicator cannot be used, and the sensitivity of the environmental water requirement (EWR) parameter. The resulting and consensus-based single metric, covering the entire globe, modelled at various temporal and spatial scales for application in LCA, is presented in details along with the interpretation and application on the rice case study. The group is proposing the result of its work and new consensus-based indicator with the expectation that it will be adopted widely and hence decrease disparity and confusion when it comes to applying the new ISO standard on water footprinting, by providing an internationally approved,

robust and simple indicator for assessment of potential impacts from water consumption.

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Characterisation of water scarcity impact on human health – development of a consensus-based model within WULCA
M. MOTOSHITA, National Institute of Advanced Industrial Sci. and
Human health is one of the impacted area of protection from water scarcity. There are several characterisation models to assess the impacts on human health caused by agricultural/domestic water scarcity. However, these models characterise the impacts on human health in different ways even though they focus on the same impact pathways. A recommended characterisation model has been developed based on consensus among method developers and stakeholders in WULCA working group of UNEP/SETAC Life Cycle Initiative. As a first step for the development, sensitivity of parameters used in effect factors of previously developed models on agricultural water scarcity (Pfister et al. 2009; Boulay et al. 2011: Motoshita et al. 2014) and domestic water scarcity (Motoshita et al. 2011; Boulay et al. 2011) were analysed and reviewed to identify critical parameters in characterisation model. The results of sensitivity analysis indicated the significance of health response factor to food/household water deficit and adaptation capacity to potential health damage. In order to test the validity of different types of health response factors and adaptation capacity, health damage due to agricultural/household water deficit was estimated based on those factors and compared with malnutrition/diarrhoea damage reported by WHO. According to the comparison of estimated and reported damage, health damage per calorie/water in deficit and inequality adjusted adaptation capacity (HDI-base) showed closer estimation of health damage to WHO report. Regarding agricultural water scarcity, food trade effects also showed high influence on the effect factor. The trade effect factor is composed of food supply dependency on domestic and imported food, as well as of the adaptation capacity through trade. Critical parameters of trade effect are identified through sensitivity analysis. The outcome of these discussions and the rice case study allowed the group to build a recommended methodology integrating the optimal options for each of these modelling choices. This recommended model is presented as an output of this consensus building within the UNEP/SETAC LCI flagship project of environmental life cycle impact assessment indicators and we expect it to improve the results of water consumption impacts in LCA and water footprinting.

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Biodiversity impacts of land use
A. Assumpcio, IRTA; L. Mila i Canals, UNEP
Land use and land use change are main drivers for biodiversity loss and degradation of a broad range of ecosystem services. Despite substantial contributions to address biodiversity in LCA, no clear consensus exists on the use of specific impact indicator(s) to quantify land use impacts on biodiversity. This lack of consensus not only limits the application of existing models, but also imposes constraints on the comparability of results of different studies evaluating land use impacts based on applying different models. This TF aims at global guidance and consensus regarding indicators and methods for the assessment of biodiversity impacts from land use in LCA. In order to identify models of particular promise for further application and development, Land use Task force has performed a review of existing indicators in and out of the field of LCA. 30 models were selected. Based on the approach used by the European Commission within the International Reference Life Cycle Data System, we grouped sets of evaluation criteria under the following categories: completeness of scope; biodiversity representation; impact pathway coverage; scientific quality; model transparency and applicability; and stakeholder acceptance. In addition, two expert workshops were organized during 2014 (San Francisco, USA, 7/11 and Brussels, BE, 18-19/11). The events included discussions centred on four key topics: (a) concept of biodiversity and modelling strategies, (b) data availability and feasibility, (c) desired characteristics of indicators, usability and consensus and (d) concerns and limitations about using biodiversity indicators in LCA. Based on outcome of expert workshops and revision conducted we could summarize that there is clearly a need to model characterisation factors in terms of both (i) local damage factor for direct land use, and (ii) regional "state and pressure" weight to reflect broader biodiversity patterns and processes surrounding the location of land use. For reasons of data availability, species richness is an obvious candidate for both local, and regional damage. However, species richness is insufficient to depict the complexity of biodiversity and ecological processes. One pragmatic way of building consensus would be to use a combination of available indicators from the reviewed models for both local and regional biodiversity damage. A rice case study is developed to test different options.

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Health effects from indoor and outdoor exposure to fine particulate matter in life cycle impact assessment
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Fine particulate matter (PM2.5) pollution has been estimated to contribute more than 7% to the total global human disease burden from 1990 to 2013 (http://healthdata.org/gbd). Ambient (outdoor) and household indoor PM2.5 exposures are reported to account for 41% and 58% of this impact, respectively, emphasizing the need to include both, outdoor and indoor exposure into overall estimates of health burdens in life cycle impact assessment. However, lacking clear guidance on how to consistently include health effects from exposure to PM2.5 in life cycle perspective, practitioners fail to report related life cycle impacts. To address this gap, a global initiative has worked on building a coupled indoor-outdoor intake fraction framework combining exposure to PM2.5 emitted indoors and outdoors with exposure to PM2.5 formed indoors and outdoors from chemical reactions. An exposure-response model derived from ambient PM2.5 concentrations is consistently combined with exposures from indoor and outdoor sources. All factors are systematically built into a model parameterized for different archetypal outdoor and indoor settings, such as specific residential and occupational settings and different urban area sizes. Model and parameters are tested in a case study on the production and processing of rice in three distinct scenarios covering urban China, rural India and U.S.-Europe. Recommendations are to use this coupled, generic framework whenever emission locations are unknown and to apply spatial models whenever emission locations are known. Our study constitutes a first step towards providing guidance on how to include health effects from PM2.5 indoor air exposures in product-oriented impact assessments.

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Improving global warming impact assessment: From recent developments in climate science to LCA practice
A. Levasseur, CIRAIG - École Polytechnique de Montréal / Chemical Engineering; F. Cherubini, NTNU / Energy and Process Engineering
In life cycle assessment (LCA), global warming impacts are usually assessed using Global Warming Potentials (GWP) for a 100-year time horizon as published by the Intergovernmental Panel on Climate Change (IPCC). In the recent past years, concerns have been raised regarding the use of appropriate modeling choices and alternative metrics have been proposed. The Global Warming Task Force of the project entitled Global Guidance on Environmental LCIA Indicators let by the UNEP/SETAC Life Cycle Initiative has performed an extensive critical review of current knowledge and limitations regarding climate metrics. Topics such as the consideration of near-term climate forcers, the inclusion of carbon-cycle and climate feedbacks in GWP, or the consideration of biogeophysical climate forcings from land use and land cover changes have been discussed. Special focus has been set toward new findings presented in the fifth IPCC assessment report, Working Group I, Chapter 8. The pros and cons of each modeling choices have been identified and recommendations have been drafted. The main line of thought is to first use more than one indicator (e.g. different time horizons, with and without carbon-cycle and climate feedbacks) to test the sensitivity of global warming LCA results to the different metric choices. If conclusions are unchanged, LCA results are robust. If they change from one metric to another, the range of results should be used to communicate about the sensitivity of LCA results to the metric choice. Metrics using different modeling choices have then been applied to a case study about the consumption of rice in three regions of the world. It has shown that LCA results may be particularly sensitive to the time horizon selected, and that the consideration of near-term climate forcers implies uncertainty and inventory data availability issues.

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Reaching consensus on cross-cutting issues
F. Verones, NTNU / Department of Energy and Process Engineering
Consistency across impact categories is important, in order to facilitate and enable comparisons across impact pathways. There are multiple issues that need to be dealt with in a cross-cutting manner and not all of them can be resolved in a simple manner. The focus of last year's work of the cross-cutting issues task force has focused on spatial aspects, normalization, uncertainty, reference states consensus for endpoint units and metrics for human health, ecosystem quality and resources, as well as a glance towards how current life cycle assessment (LCA) can be related to socioeconomic indicators. There is an unanimous consensus to keep DALY (Disability Adjusted Life Years) as endpoint indicator. We acknowledge that this does already contain a weighting, which is however internationally well-accepted. Endpoint indicators for ecosystem quality need to reflect species disappearance at a global level. There are different approaches how this can be reached and consensus is required. It is especially important that method developers provide the means to convert different units, such as PDF and PAF (Potentially disappeared/affected fraction of species). This will ensure full consistency between different impact categories. A preliminary consensus was reached that the vulnerability of different species or ecosystem types needs to be included. Models for doing so within LCA are being developed, but will need further investigation for consensus-finding. Especially important for ecosystem quality is also the discussion of reference states. It is difficult to find one common reference state across all areas of protection or all impact categories within one area of protection. We therefore propose to group impact categories in a meaningful way (e.g. based on ecosystem type affected), in order to share one common reference state. Other sub-tasks, like finding consensus on an optimal

spatial level, approaches for normalization and weighting, as well as the metric for resources are at the time of writing in discussion, without having reached a final recommendation yet. We would also like to stress that most recommendations are aimed for an immediate use. We acknowledge that LCA is a very dynamic field with many ongoing developments in terms of operational methodologies and refinements and we encourage further development and investigation that in future could lead to adapted recommendations.

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Mainstreaming life cycle thinking through a consistent approach to footprints
b. ridoutt, CSIRO
Over recent years, footprints have emerged as an important means of reporting environmental performance. Some individual footprints have become quite sophisticated in their calculation procedures. However, as an overall class of environmental metrics they have been poorly defined, having a variety of conceptual foundations and an unclear relationship to LCA. The variety and sometimes contradictory approaches to quantification have also led to confusing and contradictory messages in the marketplace which have undermined their acceptance by industry and governments. In response, a task force operating under the auspices of the UNEP/SETAC Life Cycle Initiative project on environmental Life Cycle Impact Assessment has been working to develop generic guidance for developers of footprint metrics. The initial work involved forming a consensual position on the difference between footprints and existing LCA impact category indicators. In short, footprints are deemed to have a primary orientation toward society and nontechnical stakeholders and report only on selected topics of concern. On the other hand, LCA impact category indicators have a primary orientation toward technical stakeholders and report in relation to a larger framework designed for comprehensive evaluation of environmental performance and trade-offs. The task force has also developed a universal footprint definition, as an essential prerequisite for the development of more detailed guidance. In parallel to Area of Protection, we introduce Area of Concern. In the same way that LCA uses impact category indicators to assess impacts that follow a common cause-effect pathway toward Areas of Protection, footprint metrics address Areas of Concern. The critical difference is that Areas of Concern are defined by the interests of stakeholders in society rather than the LCA community. In addition, Areas of Concern are stand-alone and not part of a framework intended for comprehensive environmental performance assessment. The Area of Concern paradigm is needed to support the development of footprints in a way that fulfils their distinctly different purpose. Accordingly, footprints are universally defined as metrics used to report life cycle assessment results addressing an Area of Concern. The task group is now actively working on detailed guidance. One priority topic is the acceptable use of aggregation and weighting where the Area of Concern requires the use of multiple impact assessment models.

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Product environmental footprint (PEF): improving life cycle impact assessment
M. Galatola, European Commission / DG Environment
Regarding the European policy context, the presentation will continue by presenting the consensus process initiated by the European Commission, aiming at covering land use related impact at midpoint, water related impact, respiratory inorganics and resource depletion to be used in the context of the Product Environmental Footprint (PEF) studies. Progress on this impact assessment harmonization process will also be presented in this session.

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Life cycle impact assessment models for PEF: assessment of models and applicability challenges
S. Sala, European Commission - Joint Research Centre / Sustainability Assessment unit; R. Pant, European Commission / Institute for Environment and Sustainability
Regarding the European policy context, the presentation will continue by presenting the consensus process initiated by the European Commission, aiming at covering land use related impact at midpoint, water related impact, respiratory inorganics and resource depletion to be used in the context of the Product Environmental Footprint (PEF) studies. Progress on this impact assessment harmonization process will also be presented in this session. The session will end by a stakeholder presentation from industry and government workshop representatives and general feedback from session participants.

The sustainability of wine production in Europe

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Ecosystem services and soil biodiversity in French vineyards
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Essential ecosystem services provided by viticultural landscapes result from diverse communities of above- and belowground organisms and their interactions. As a result of management intensification in the last century several ecosystem services were affected leading to high rates of soil erosion, degradation of soil structure, soil fertility, contamination of groundwater and decrease of longevity of vines etc. Here we report how management intensity affects biodiversity of aboveground and belowground biota as well as their impact on ecosystem services in terms of regulating services in the agro-ecosystem, provisioning services and cultural services. Some alternative techniques and conservation measures that have the potential to mitigate the negative effect on biodiversity were introduced in some wine regions, thereby restoring ecosystem services. Furthermore, the impact of landscape structure on biodiversity and ecosystem services in vineyards is currently investigated in the Biodiversa project “VineDivers”.

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Footprinting the sustainable wine production in Italy
E. Capri, Universitá Catolica del Sacre Cuore

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Study of the variability of a vineyard sensitivity to fungus diseases in order to adapt chemical input: a priori zoning of Physiological Behavior Units (PBU) using precision viticulture techniques
m. raynal, B. DELFOUR, C. DEBORD, M. VERGNES, A. BENNABI, Institut Français de la Vigne et du Vin; M. GEORGES, R. FULCHIC, J. SERVANT, Château Léoville Las Cases
The systemic analysis of the performance of a vineyard at the scale of a wine-making exploitation is made possible by the use of sensors stemming from so called precision techniques, which allow precise and exhaustive geo-located measures. The aim of our study is to exploit this kind of data and evaluate their information using geographical information systems (GIS) and crossing different layers representing characteristic and independent variables of the production system. The goal is then to elaborate an à priori zoning, likely to explain variations of the physiological development of vines and possible differences of the plants susceptibility to fungus diseases. The study is based on the combination of two maps established on the property of Chateau Léoville Las Cases in the Medoc area of the Bordeaux vineyard (France). These maps represent the behaviour of the two compartments, soil and plant, respectively determined by means of electric resistivity (R) and biomass index (B) measures. Three levels - low, medium, high- are defined for each type of data. The combination of these indicators allows the elaboration of 9 classes of islets, named Physiological Behaviour Units (PBU), whose distribution is bounded by the GIS on the whole vineyard. Six of these nine PBU were selected by exclusion of the medium class of the biomass index. Each PBU is replicated twice, thus establishing an observation device of 12 PBU likely to identify differences in terms of physiological development and disease susceptibility. For this purpose, treated and non-treated zones were delimited for each PBU, and a weekly monitoring of these areas has been performed during the 2014 and 2015 crop years. The first years' results of the study show that the PBU concept proposed seem to correlate with some of the significant variations observed for physiological and sanitary criterions.

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Guided discussion: sustainable wine production: opportunities, obstacles and the path forward
S.E. Apitz, SEA Environmental Decisions Ltd

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Summary, conclusions & next steps
S.E. Apitz, SEA Environmental Decisions Ltd; S. Delrot, University of Bordeaux1; F. Martin-Laurent, INRA / Agroecologie

Biodegradability assessments of organic substances and polymers

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Chemical analysis and measurement of biomass to enhance interpretation of hydrocarbon solvent biodegradability tests
C. Hughes, Shell International Limited / Shell Health; D. Brown, Shell Global Solutions International B.V.; G. Whale, Shell Health / Shell Health
Biodegradability is a key parameter for environmental hazard and risk assessment of chemicals. This is typically assessed experimentally following the OECD 301 A-F series of ready biodegradability tests in the first instance. These tests measure the biodegradation of a chemical under stringent conditions, and as such are considered a screening test for readily biodegradable substances. The results of these tests are used extensively both within a regulatory context and to support marketing claims on product biodegradability. However, these methods do have

their limitations, particularly when they are used to assess the biodegradation of complex, volatile and/or poorly water soluble substances. Recent investigations with GTL products have highlighted these issues. In particular, the inclusion of specific chemical analysis in biodegradation studies (not always a guideline requirement) has shown that disappearance of test substances from biodegradation systems is often far greater than is suggested by respirometric measures alone. This is indicative that either the material has been lost from the test system through abiotic processes (e.g. volatilisation), or that it has not been completely mineralised (e.g. it has been used as a carbon source to increase microbial biomass). The OECD 301F test was found to be more suitable for testing such substances than 301B, because it utilises a sealed system and therefore limits losses due to volatility. In this study a number of hydrocarbon solvents have been tested using OxiTop®-C test systems, following the OECD 301F test method. In addition to measuring biodegradation by biological oxygen demand (BOD), additional techniques have been incorporated to enhance the interpretation of results. Analysis by GC-FID and by TIC/TOC has been conducted to assess the presence of residual test substance and other metabolites, and the increases in microbial biomass have been measured to give an indication of the amount of carbon used by the inoculum to increase its biomass, rather being completely mineralised. In addition, abiotic controls have been run to assess removal from processes other than microbial degradation. Using this combination of additional measures and analyses, it is possible to account for a greater percentage of the total material tested, and therefore to improve the interpretation of these tests.

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Approaching chemicals' persistence through a new strategy of use of RBT tests
F. Brillet, University of Nantes / Microbiology; A. Maul, Univeristy of Lorraine / Applied Statistics; M. Durand, University of Nantes / UMR CNRS GEPEA CEBAC Laboratory; T. Gerald, University of Nantes / Microbiology
Approaching chemicals' persistence through a new strategy of use of RBT tests Brillet François[1], Maul Armand[2], Durand Marie-José[1] and Thouand Gérald[1] [1]Université de Nantes, UMR CNRS 6144 GEPEA, IUT Génie Biologique, La Roche sur Yon, France. [2]Université de Lorraine, LIEC-UMR CNRS 7360, Metz, France gerald.thouand@univ-nantes.com With millions of organic chemicals released every day into our environment, Europe and other continents are confronted with increased health and environmental problems. Even if strict regulations are imposed upon industry to ensure that they prove the harmlessness of their substances during marketing authorization, not all testing procedures are designed to cope with the complexity of the environment. This is especially true for the evaluation of persistence through biodegradability assessment guidelines. Our new approach has been to adapt *in vitro* biodegradability assessment to the environmental conditions and model the probability for a biodegradation test to be positive in the form of a logistic function of both the temperature and the cell density. Here, a proof of this new concept is proposed with the establishment of 3D biodegradability profiles of 6 chemicals (including the controversial glyphosate) between 4 to 30 °C and 10^4 to 10^8 cells ml^{-1} as can be environmental compartments in time and space. The results show a significant increase of the predictive power of existing lab scale tests and the creation of new indicators to quantify environmental persistence. Key words: Biodegradability Assessment, Environmental Persistence

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Screening for Persistence: Tools to Determine Mineralization and Estimate Kinetics in Water, Water-Sediment and Soil
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Standardized biodegradation tests provide data that are required for persistence assessment (e.g. under REACH). In a first step, clearly non-persistent substances are identified within a persistence screening based on results from water-only ready biodegradability tests (RBTs) and QSAR models. However, RBTs providing quantitative biodegradation data for sediment and soil are still lacking. Hence, predictions of degradation rates and half-lives of organic compounds by QSAR models are mainly based on qualitative experimental biodegradation data related to water-only test systems. Moreover, half-lives related to water are extrapolated to soil and sediment by multiplication with default factors. Within this study, the existing experimental screening test method to determine biodegradation in water according to OECD 301C (MITI, Ministry of International Trade and Industry, Japan) was modified to develop new test systems on the screening test level for water-sediment (WSST, Water-Sediment Screening Tool) and soil (SST, Soil Screening Tool). The test systems were applied to determine mineralization rates and kinetics of 15 organic compounds in water, water-sediment and soil (45 tests in total). Resulting degradation curves were fitted by different non-linear regression models and kinetic parameters were derived using the regression model with the best fit. The experimental results were verified based on (i) validity criteria according to OECD 301, (ii) reproducibility of results, and (iii) comparability with biodegradation data from well-established test methods. In addition, options how the determined mineralization rates and kinetics could be applied within a persistence screening are presented. The new screening-test systems WSST and SST proved to be suitable tools to determine reproducible and sound quantitative biodegradation data including biodegradation kinetics for water-sediment and soil, respectively, that could be applied in the regulatory context (e.g. within a persistence screening). The observed substance-specific variation of biodegradability in different environmental compartments provides strong evidence that extrapolation of half-lives from water to sediment and soil by use of default factors should be avoided. Beyond that, the test systems can be used for building up a database of screening-level biodegradation information across major compound classes, which can serve as reference set for subsequent research into respective in silico models.

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Identifying limitations of the OECD water-sediment test (OECD 308) and developing suitable alternatives to assess persistence
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The OECD guideline 308 describes a laboratory test method to assess aerobic and anaerobic transformation of organic chemicals in aquatic sediment systems and is an integral part of tiered testing strategies in different legislative frameworks for the environmental risk assessment of chemicals. Over the years, several shortcomings of the OECD guideline 308 have been identified and its usefulness for persistence and exposure assessment has been questioned. On October 6, 2015, a final stakeholder workshop of the Cefic-funded project “LRI ECO18 – Improved strategy to assess chemical persistence at the water-sediment interface” was held. The goal of this workshop was to summarize stakeholder perspectives on the status quo of both OECD guidelines 308 and 309, and to complement this with a dissemination of the findings of the project LRI ECO18. In this presentation, the main outcomes of the workshop will be presented, i.e., (i) sharing of industry and regulatory experience with OECD 308 and 309 in different regulatory contexts, (ii) identification of major issues and knowledge gaps with respect to OECD 308 and 309, and (iii) presentation of main outcomes and recommendations from LRI ECO18 project [1-3]. [1] Honti M, Fenner K. 2015. Deriving Persistence Indicators from Regulatory Water-sediment Studies – Opportunities and Limitations in OECD 308 Data. Env Sci Technol 49: 5879-5886. [2] Shrestha P, Junker T, Fenner K, Hahn S, Honti M, Bakkour R, Diaz C, Hennecke D. 2015. Simulation studies to explore biodegradation at the water-sediment interface – From OECD 308 to OECD 309. In preparation. [3] Honti M, Junker T, Hennecke D, Hahn S, Shrestha P, Fenner K. 2015. Bridging Across OECD 308 and 309 Data in Search of a Robust Transformation Indicator.In preparation.

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Biodegradation of squalane by a Pseudomonas aeruginosa strain and bioavailability considerations
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Very few studies have reported bacterial degradation of the long chain length, acyclic isoprenoid, squalane (2,6,10,15,19,23-hexamethyltetracosane) although there are several reports on biodegradation of smaller chain length isoprenoids, such as pristane and phytane. The low aqueous solubility and structural complexity limits the bioavailability of branched alkanes to most strains. This study reports squalane biodegradation by a *Pseudomonas aeruginosa* strain RS1 isolated from refinery sludge that could utilize it as sole source of carbon and energy after a long acclimatization period was provided. It exhibited specific growth rate in the range 0.19-0.22 h^{-1} over the squalane concentration range of 250 - 750 mg/L in Bushnell-Haas mineral media. The maximum extent of degradation (67%) was achieved after 96 h for squalane concentration 250 mg/L where abiotic loss was less than 20%. Negligible extracellular release of biosurfactants was observed. Contact angle measurements revealed that the cell surfaces of squalane grown cells were moderately hydrophobic, thus, indicating direct interfacial uptake of squalane. However, the adherence to n-hexadecane and squalane in the bacterial adhesion to hydrocarbon (BATH) assay was unexpectedly low (less than 30%). This apparent anomaly may be explained by the fact that BATH assay is not a true measure of hydrophobicity since it is likely to be affected by solution phase interactions, such as, electrostatic interactions. A similar mechanism was employed by this strain for degrading the aromatic hydrocarbon, pyrene, at relatively high rate compared to other pyrene degraders This is the first study reporting degradation kinetics and bioavailability considerations for squalane uptake and thus provides insight on degradation of branched alkanes in petroleum.

Mercury Biogeochemistry and Policy

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Mercury concentrations in suspended particulate matter, water and mud-sediment up and downstream from old and recent gold mining sites in French Guiana

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Although the use of mercury (Hg) for gold mining has been officially banned in French Guiana since 2006, there are still reasons to be wary of its occurrence in river water and its impact on local populations. In fact, gold-mining generates intense soil erosion and high turbidity in the rivers downstream from mining sites. This contributes to Hg pollution and dissemination both via the remobilization of Hg naturally present in the soils and the release of metallic Hg from the gold recovery process. In the affected rivers, Hg transport and speciation is mainly controlled by the suspended particulate matter (SPM) rather than remaining dissolved. The aim of this study is to understand and quantify the fate of this SPM-associated Hg along the Boulanger River affected by old and new gold mining activities. Furthermore we provide recent data (2012) essential to evaluate the efficiency of legal gold mining companies in restoring and re-planting exploited zones to control erosion, river turbidity and SPM transport. Inorganic Hg(II), monomethylmercury (MMHg) and turbidity measurements were measured in water, SPM and mud-sediment collected along the Boulanger River, from the spring down to its confluence with the Orapu River. The intensity of gold-mining in this area has been up and down through the last century and presents contrasting conditions with old and recently exploited areas. Dissolved Hg (HgD) levels were low (1-2 ng.L^{-1}) and consistent with previous data known for the Amazonian area. Dissolved MMHg concentrations represented up to 8% of HgD which is higher than previous results (around 2%). Some relationships occurred between Hg concentrations in mud-sediment and turbidity values which both decreased downstream from the mining sites, then stabilized at 4-5 km further down. Hg contents in the mud-sediment show the impact of old and new gold mining practices. These results show that even though Hg use was banned from French Guiana 6 years ago, present gold mining practices still release significant amounts of Hg into the rivers. Survey intensification in French Guiana river systems should provide a more complete data set, essential to change gold mining practices and to reduce SPM and Hg contamination of the aquatic environment.

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In situ biomonitoring of mercury bioavailability and transfer in a former gold mining area (French Guiana).

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Gold mining activities have a considerable impact on the entire ecosystem, including terrestrial and aquatic compartments. Beside the physical degradation of soils and river network, artisanal small-scale gold mining also leads to the release of mercury (Hg) in the environnement. The sources of Hg are both anthropogenic (from fine gold particles amalgamation) and geogenic (from weathering of parent rocks and long-term atmospheric deposition). Mercury contamination of the aquatic food web constitutes both an environmental and sanitary risk since local populations largely feed on topchain predator fishes which exhibit high Hg concentration. Among Hg species, monomethyl-Hg (MMHg) represents the most bioaccumulative and toxic form. The objective of the present study was to investigate the bioavailability and the transfert potential of Hg in a former gold mining area in French Guiana using chironomid larvae, key organisms of the aquatic ecosystem functionning and food chains. Hence, in an active biomonitoring campaign, we investigated (i) inorganic-Hg (IHg) and MMHg toxicokinetics in larvae encaged in the field and (ii), by mean of designed exposure devices, the contributions of water and sediments to both IHg and MMHg uptake. The study site was a former goldmine flat of the Combat Creek catchment (French Guiana) exploited until 2010. We used calibrated fourth instar larvae of *Chironomus riparius* bred in the laboratory (IRD Cayenne) under controlled conditions. Larvae were exposed in two different types of cages allowing the contribution of water and sediments in IHg and MMHg bioaccumulation to be estimated. Mercury speciation was conducted (GC-ICP-MS) both in unsoluble (exoskeleton, gut content and granules) and soluble (cytosol) fractions. Although IHg did not accumulate, MMHg was bioavailable to chiromid larvae and mainly accumulated in the cytosolic compartment. Moreover, sediments appeared as an important source of MMHg as testified by the higher uptake rate modelled in larvae exposed to both water and sediments compared to water exposure only (0.57 and 0.18 ng.g^{-1}.d^{-1}, respectively). Hence, the active biomonitoring using *C. riparius* larvae allowed to efficiently estimate MMHg bioavailability and its potential of transfer in the trophic web since the fraction stored in the cytosolic compartment can be considered as trophically available to most, if not all, predators.

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Linking variation in natural solar radiation with seasonal methylmercury dynamics in freshwater lake systems

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Methylmercury bioaccumulation in biota is a serious concern for mercury sensitive ecosystems far from direct point sources of pollution. Low buffering capacity in soils and high atmospheric deposition of mercury and acid rain from anthropogenic sources can enhance the solubility and transport of metals into waterways. Plentiful wetland environments and well-mixed lakes make studying the processing controlling methylmercury concentrations in water dynamic and complex. While much is know about mercury methylation in freshwater, much less is known about demethylation. The loss of methylmercury from the water column of lakes is dependent on several processes including adsorption, deposition to sediments, and demethylation. Microbial demethylation in water columns is very slow, therefore a daytime photodemethylation facilitated by solar radiation can dominate methylmercury removal from the water column. To better quantify the photodemethylation potential within lakes we must determine a) the variation in photoreactive compounds such as dissolved organic matter (DOM) and iron (Fe) and b) the availability of solar radiation with depth in water columns. Freshwater lakes were chosen in Kejimkujik National Park (44.23°N, 64.13°W) to include a wide range in dissolved organic carbon (DOC) and Fe concentrations. Water samples were collected over 3 years and analyzed for ultraviolet (UV)-visible absorbance, DOC, dissolved ions, total mercury, and methylmercury concentrations. Floating sensors for UV, photosynthetically active radiation (PAR), and temperature were also installed in two lakes of contrasting DOC concentrations. The depth of 95% UV attenuation was 40-50 cm in the lower carbon lake compared to 10-20 cm in the higher carbon lake. The effect of rainfall on UV attenuation was also less in the lower carbon lake compared to the higher carbon lake, most likely due to a difference in catchment area. Seasonal alterations to the solar angle of incident radiation strongly controlled the amount of solar radiation entering lake water surfaces and therefore outlined a possible photoreactive season or period within each year. These observations suggest that photodemethylation of methylmercury in lake water columns may be limited to a short period of approximately 4 months a year at 44°N. Overall, these field observations provide fine resolution solar radiation data and excellent temporal resolution over 3 years for mercury and carbon cycling.

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Dietary transfer of Hg from Elodea nuttallii to Gammarus fossarum

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In the aquatic environment, the organic form of Hg, methyl-Hg (MMHg) and inorganic Hg (IHg) are both toxic, but MMHg has been shown to biomagnify through food chain while IHg does not. Nonetheless, the precise factors controlling the transfer of Hg from primary producers to heterotroph consumers remains poorly understood. Macrophytes are key organism of shallow aquatic environment in which MMHg is produced, and have been identified as having a role in Hg transfer to food webs. The objective of this study was therefore to assess how the compartmentation of IHg and MMHg in a representative macrophyte, *Elodea nuttallii*, affects their bioaccumulation through dietary transfer in *Gammarus fossarum* chosen as surrogate species for aquatic herbivores and decomposers. Bioaccumulation of Hg in *E. nuttallii* was higher for cell wall than intracellular in line with previous studies showing an important role of binding of Hg in cell wall in *E. nuttallii*. Our data suggest no substantial demethylation or methylation during bioaccumulation in *E. nuttallii in planta* or in water. THg and MMHg concentrations increased in Gammarids in correlation with concentration found in *E. nuttallii*. MMHg was more transferable than IHg, based on THg, but a significant part was demethylated during the feeding, while no methylation was observed in the IHg exposure. Cell wall results in higher concentrations than intracellular, suggesting that Hg in cell wall in form of MMHg and IHg was significantly assimilated by Gammarids. Significant uptake fluxes were estimated by the first-order model: uptake fluxes in Gammarids were higher for cell wall than intracellular, while similar uptake rates were observed for IHg and MMHg. In conclusion, *G. fossarum* is able to feed on *E. nuttallii* and accumulates Hg from **intracellular** and cell wall **compartments**. In line with literature, when looking at THg, consumption of MMHg-exposed plants leads to slightly higher accumulation of Hg than IHg-exposed plants, but we observed a significant demethylation of MMHg in Gammarids *vs* plants. Opposite to literature, we did not observed a significant biomagnification of MMHg. In sum, although the subcellular metal distribution is determinant for many consumers that are unable to assimilate the insoluble fraction of cell **walls**, the digestive strategies to handle food of the consumer is also important. Differences in primary producers and consumers species composition may significantly influence the fate of Hg in food webs.

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Concentrations of mercury in porbeagle shark Lamna nasus

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Concentrations of mercury in three tissues of porbeagle shark *Lamna nasus* (n = 33) were determined. While all specimens exceeded OSPAR's Background Assessment Concentrations of 0.035 mg kg^{-1}, Hg concentrations in either the red or white muscle that exceeded European regulations for seafood were observed in one-third of specimens. Hg concentration, however, increased with length, and all fish >195 cm had concentrations >1.0 mg kg^{-1}, with a maximum observed value of 2.0 mg kg^{-1}.

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Spatial and temporal monitoring of total mercury and monomethylmercury in fish from European freshwaters
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For the implementation of the European Water Framework Directive (2000/60/EC) an environmental quality standard (EQS) of 20 µg/kg mercury (Hg) in fresh weight of aquatic organisms was derived (EU Directives 2008/105/EC, 2013/39/EC). Because of their differing chemical behavior and ecotoxicity (e.g., secondary poisoning of predators by MeHg) inorganic Hg and organic Hg (mainly monomethylmercury, MeHg) should be evaluated differently. However, in aquatic organisms only determination of total mercury concentrations is required for the WFD compliance monitoring. Here we quantified also the MeHg levels in the fish samples to allow a better risk assessment of the Hg residues. From a previous monitoring program bream (*Abramis brama*) collected from different European freshwaters were available. In the period 2007-2013 the rivers Tees/UK, Mersey/UK (no sampling 2009-2011), Götaälv/SE (no sampling 2009-2011), Western Scheldt/NL, and Rhône/FR as well as a lake (Belau/DE) were covered. Pooled muscle tissue samples of 15 fish per site were homogenized and analyzed for total Hg with a Direct Mercury Analyzer as well as for MeHg by SID-GC/ICP-MS (species isotope dilution-gas chromatography - inductively coupled plasma-mass spectrometry). The results of the total Hg analyses of the fish samples from European freshwaters were then compared to the long-term freshwater fish monitoring data of the Environmental Specimen Bank operated by the GermanFederal Environment Agency. Data reveal that both for the fish from the European as well as from the German river sites EQS were clearly exceeded. Only data for the one German lake site investigated revealed total Hg levels in the range of the EQS in recent years. Regarding the MeHg fraction of the total Hg literature data could be confirmed which report high MeHg fractions in the range of 70-100 %.

Mechanistic toxicology of engineered nanomaterials: state of the art and future perspectives (I)

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Oxidative stress response of the aquatic macrophyte Hydrilla verticillata exposed to nanoparticulate and bulk TiO2
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Nanotechnology is a rapidly growing industry with manufactured titanium dioxide nanoparticles (TiO_2-NPs) being one of the most commonly employed metal oxide NPs. Due to increasing production volumes concerns about the fate and behaviour of nano-TiO_2 in the aquatic environment have arisen, as water reservoirs will be the final destination of TiO_2-NPs. Currently, there is emerging research activity concerning the ecotoxicology of TiO_2-NPs in aquatic ecosystems. However, biochemical studies investigating the effect of TiO_2-NPs on oxidative stress related parameters in aquatic macrophytes are scarce, despite their important role in aquatic habitats. In the present study, oxidative stress effects in *Hydrilla verticillata* caused by TiO_2-NPs of different cristallinity status were investigated and compared to a bulk sized counterpart. Macrophytes were exposed to different concentrations of TiO_2-NPs (0, 0.01, 0.1, 1, 10 mg/L) for 24 h, and thus experimental setup included currently predicted levels of nano-TiO_2 in surface waters. Additionally, the investigated TiO_2-NPs contributed varying crystalline status (anatase, rutile, P25-mixed phase) to assess a potential influence of crystalline phases on oxidative stress responses. As oxidative stress related parameters the level of hydrogen peroxide (H_2O_2), reduced and oxidized glutathione (GSH and GSSG) and activities of the antioxidative enzymes peroxidase (POD), catalase (CAT) and glutathione reductase (GR) were measured. Whereas POD was not considerable activated in this study, results imply an activation of the enzymatic defense system, as increased CAT and GR activities were observed. No significant changes in enzyme activities were assessed for the treatments with bulk TiO_2 and moreover, such exposed plants exhibited lower enzyme activities at all concentration steps, suggesting a nano-specific influence on antioxidative defense mechanisms in *H. verticillata*. All TiO_2-NP concentrations dropped the GSH/GSSG ratio, indicating a high GSH-dependent metabolic activity to protect against the destructive effects of reactive oxygen species (ROS) generated during nano-TiO_2 exposure. Furthermore, the glutathione status seems to be a sensitive marker for changes in the cellular redox state of macrophytes. As the level of H_2O_2 was solely elevated after exposure to 10 mg/L of P25, adaptive metabolic mechanisms of *H. verticillata* are probably able to cope with environmentally relevant concentrations of TiO_2-NPs.

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Does a coating matter? Antioxidant enzymes activities in the water flea Daphnia magna exposed to modified copper oxide engineered nanomaterials.
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Production of reactive oxygen species (ROS) has been described as a general pathway of toxicity induced by various metal based engineered nanomaterials (ENMs) and other chemicals leading to oxidative stress Coating-related changes in three antioxidant enzymes' activities in the freshwater flea *Daphnia magna* were assessed, based on information obtained in acute and chronic toxicity tests. Experiments on biochemical responses following exposures to different CuO ENMs were focused on the activities of catalase (CAT), superoxide dismutase (SOD) and glutathione-S-Transferase (GST) after 2, 6 and 24 hours of exposure. To minimise variability in the response to the analysed CuO ENMs, a single laboratory clone of *D. magna* (clone GG4) cultured at Heriot-Watt University, was selected for this study. Cultured juveniles aged 5-8 days were used for biochemical analysis: 450 for CAT, 450 for SOD, 450 for GST and protein analyses, with three replicates per treatment. The animals were exposed to sublethal CuO ENM concentrations, corresponding to EC50 values obtained after 48 hours of exposure. Enzyme activity responses varied across the ENM panel. SOD, CAT and GST, which are considered the most important antioxidant enzyme systems in invertebrate species, showed different responses across the different surface modifications and time of exposure. After two and six hours of exposure *Daphnia* juveniles exposed across the ENM panel did not show significant increase in SOD activity. After 24 hours of exposure, SOD activity significantly increased for animals exposed to CuO, CuO-COOH and CuO-NH_3 ENMs. After 2 hours no significant differences between treatments were observed for the GST activity across all tested chemicals. The GST activity significantly increased after exposure to CuO-COOH and CuO-NH_3 ENMs for 6 hours, and for CuO-PEG after 24 hours exposure. No significant effects on CAT were observed after 2 and 24 hours of exposure; only CuO-PEG ENM showed marginal but not significant increase in CAT activity after 24 hours. Significantly increased CAT activity was observed following exposure to unmodified copper oxide and CuO-NH_3 ENMs after 6 hours. The most sensitive endpoints after 6 hours were CAT and GST assessments and SOD, after 24 hours exposure. Based on the above results it is apparent that ROS play an important role in the toxicity pathway observed, and the pattern observed depends on the CuO ENMs surface modification and time of exposure.

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Secreted protein eco-corona mediates uptake and impact of 'next-generation nanoparticles' on Daphnia magna
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Nanoparticles (NPs) have at least one external dimension between 1-100nm. The small size of NPs gives them a large surface area to volume ratio providing them with exclusive properties that differ from bulk material of the same chemical composition. As a result, these unique materials have found numerous applications in industrial fields leading to their inevitable release into the environment during use or at the end of their life cycle. NPs are known to adsorb molecules such as proteins from their environment, to form an eco-corona, which ultimately changes the NP identity and how it interacts with organisms. Secretion of biomolecules is a well-established component of both intra-species communication and predator-prey response in aquatic food-chains and therefore the impact of such secretions on NP uptake and ecotoxicity must be investigated. Here we report some initial studies, using model spherical polystyrene NPs which mimic plastics that degrade slowly in the environment, resulting in build-up of nano-plastic in fresh water systems. We also look at various shaped and charged 'next-generation NPs' including gold NPs with different morphologies whose release into the environment is increasingly likely due to the heightened use of these NPs in medical systems. Finally, we look at several novel perovskite NPs, which are increasingly used in solar cells. For all NPs, the impact of the secretion of biomolecules by *Daphnia magna* on the NP uptake, retention and toxicity was assessed. Interestingly, in all cases studied to date, a pre-formed eco-corona from *D. magna* secreted biomolecules resulted in enhanced NP toxicity, related to enhanced uptake and retention of the particles, and in some cases to enhanced oxidative stress.

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Comparative evaluation of acute and chronic ecotoxicity of Copper Oxide nanoparticles on the pond snail Lymnaea stagnalis

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Nanotechnology is a rapidly developing field in the 21st century, and commercial use of nanomaterials for novel applications is increasing exponentially. Copper oxide nanoparticles (CuO NPs) are frequently employed for their antimicrobial properties in antifouling paints. Their extensive use can contaminate aquatic ecosystems. The objective of this study was to evaluate and compare the aquatic toxicity of CuO NPs through acute and chronic toxicity tests with different life stages of the snail *Lymnaea stagnalis*, a representative organism of the benthic ecosystem. Acute waterborne exposure was focused on the evaluation of the acute lethal toxicity of CuO NPs to juveniles (7-9 day old) of the snail *L. stagnalis* exposed for 96h at 20°C in a static experiment, either in the nano form of CuO NPs or ionic form, as $CuSO_4*5H_2O$. The LC50 value estimated in tests with $CuSO_4$ ($LC50_{96h}$ = 5.7 µg L^{-1} Cu) was 400 fold lower than that obtained for the tests with CuO NPs ($LC50_{96h}$ = 2500 µg L^{-1} Cu). Chronic toxicity tests aimed to investigate the effects of exposure CuO NPs on the reproduction to *L. stagnalis*. Young adult snails(22±2mm) were exposed to Cu as CuO NPs at 20°C for 30 days in a semi-static experiment. Endpoints such as: mortality, growth and behaviour alteration were also evaluated along with the reproduction parameters. $LC10_{30d}$ and $LC50_{30d}$ values estimated were respectively, 230 µg L^{-1} Cu and 480 µg L^{-1} Cu, indicating a 5 fold higher toxicity than the acute test. Additionally, exposure to CuO NPs showed significant effects ($p< 0.001$) on the growth and reproduction parameters relative to the control. Behavioural changes, such as respiration behaviour, were also observed in the Cu treatments. The experiments' results demonstrate a time-related increasing toxicity of CuO NPs on *L. stagnalis*, emphasizing the need for more chronic study to accurately evaluate the impact of nanomaterials in the real environment. Furthermore, long-term experiments using juveniles *L. stagnalis* exposed to CuO NPs are ongoing, evaluating growth and time-related expression profiles of antioxidant enzymes and heat shock proteins response in snails to thermal shock. Acute and chronic tests assessing the toxicity of safe-by-design CuO NPs and their fragmented products (FP) on *L. stagnalis* will be performed, applying the same experimental design used for evaluate the toxicity of the pristine CuO NPs. This research project is funded by the European FP7 project SUN "Sustainable Nanotechnologies".

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Bioavailability of metal nanoparticles in a sediment dwelling organism: a study of transdermal and oral routes of uptake
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Metal engineered nanomaterials (ENMs) are an emerging pollutant considered to be of risk to aquatic environments due to their inherent reactivity and high global production volumes. The behaviour of metal ENMs in aquatic sediment systems is dominated by transformations including aggregation, complexation with organic matter and in some cases dissolution of metal ions. Investigating ENM behaviour in sediments requires novel combinations of separation and microscopy techniques. This will allow us to correctly interpret the results from studies into the bioaccumulation of ENMs in benthic species. Using a combination of centrifugal ultrafiltration and Asymmetric flow Field Flow Fractionation (AF4) the size distribution and dissolution of both ceria (CeO2NPs) and silver (AgNPs) nanoparticles was followed in a model sediment system over 6 days. The aim was to compare uptake through transdermal or oral routes. Two commercially relevant Ag NPs were chosen as test materials: one stabilised sterically with PEG (mono mPEG phosphonic acid ester), the other with an electrostatic stabiliser, citrate. The NPs had a primary particle size of 4-10 nm. A 5 day bioaccumulation exposure was conducted using the sediment dwelling oligochaete worm Lumbriculus variegatus. Organisms were either actively feeding (uptake through transdermal and oral ingestion) or non-feeding, achieved by utilising the species' natural mode of reproduction by clonal fragmentation, to yield non-feeding clones. Centrifugal ultrafiltration examined partitioning of CeO2 and AgNPs between the solid and liquid phases of the sediment and NP dissolution. AF4 was used to investigate the size distribution and preferential heteroaggregation between the CeO2 and AgNPs and other natural colloids present in the sediment pore waters. Results demonstrate that for CeO2 NPs, dissolution does not occur and there was no uptake of nanoparticles across transdermal pathways. Coating type (electrostatic or steric) also made no difference to bioavailability of these particles through ingestion. All three NP treatments were significantly more bioavailable than either the geogenic Ce present naturally in the sediments or micron sized CeO2. Results for Ag NPs are ongoing and will be reported in full during the presentation. Results will be discussed in the context of the transformations that the nanoparticles have undergone and their interactions with other natural colloidal materials in the sediments.

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Effects of iron nanoparticles on primary cultures of human bronchial epithelial cells
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Airways constitute the major route of exposure to atmospheric particulate pollutants. Furthermore, inhalation of fine and ultrafine particles contributes to their rapid transfer to the lung. Their size allows them to reach the deep lung, and the smallest of them can be transferred to the entire human body. Recent studies have demonstrated that exposure to atmospheric particulate was associated with an increased risk of morbidity and mortality related to respiratory and cardiovascular diseases. Indeed, with the complexity of the bronchial tree, inhaled ultrafine particles are in regular contact with lung epithelial cells and may favor the emergence of chronic inflammatory diseases (asthma, COPD, etc) With the emergence of nanotechnology and the increasing use of nanoparticles, a question emerges: "what are the effects of this nanoparticles on health?" The specific properties of nanoparticles such as their smaller size, larger surface area and higher reactivity are major points of concern. Consequently, an essential step in nanoparticle study is their physicochemical characterization in their pristine state, or suspended in an aqueous medium. In the present study, we aim to investigate the lung effects of iron-engineered nanoparticles (representative of industrial smoke emitted by metallurgical industries). After characterizing these particles at the physic-chemical level and verifying their capabilities to penetrate lung cells using transmission electron microscopy, we assessed their cytotoxicity on primary lung cells and their capacity to modulate gene expression and oxidative stress. After 6-h exposure of primary cells to low dose of iron-engineered nanoparticles, we highlighted expression modulations of genes involved in inflammation but we failed to detect a significant induction of oxidative stress.

Soil and water contaminants: evaluation, biomonitoring and bioindicators for effective management (I)

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Which pollutant should we be measuring? - Broad spectrum screening provides multiple answers in a single run
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There is still little information on which chemical pollutants are present in the South African environment. To prioritise economic investment in expensive analytical techniques, valuable information should first be gained from broad spectrum screening. A broad spectrum analytical screening technique was developed for the analysis of non-polar lipophilic compounds from abiotic and biotic matrices using comprehensive two- dimensional gas chromatography time of flight mass spectrometry (GC×GC-TOFMS). Once a specific compound class is identified, the method can then be used for quantification. This provides a powerful tool in optimising the amount of data that can be generated within a single analysis. A LECO Pegasus IV, comprehensive two-dimensional gas chromatography coupled to time of flight mass spectrometry (GC×GC-TOFMS) system was used with a non-polar Rxi®-5Sil MS primary column and a mid-polar Rxi®-17SilMS secondary column. The developed method was tested on sediment samples extracted using selective accelerated solvent extraction. Non-target compounds in complex samples can be provisionally identified due to the predictable compound grouping within the chromatogram (based on physiochemical properties) and full mass spectra collected. The analysed sediment samples from industrial areas indicated the presence of various PCBs, PAHs, PAH derivatives, as well as chlorinated PAHs, steranes , hopanes, and thiophenes associated with petrochemical contamination. In agricultural areas terpernoids, terphenyls and PAHs were prevalent with various pesticides. PAH, PCB and OCP concentrations were quantified successfully using the data collected in the same analytical run. This analytical technique allowed petrochemical contamination to be identified even though the initial focus of the study was PAHs, OCPs and PCBs. Petrochemical contaminants can be of concern to human and environmental health, especially PAHs and related compounds that are known human carcinogens. This data can then be used to justify a project focused on the presence of petrochemical contaminants in the South African environment.

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Raman SERS monitoring of Acetylcholinesterase activity for the detection of pesticides
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Pesticides are well-known chemical compounds highly toxic for health and environment with important economic consequences. Usually various methods are implemented to detect pesticides such as gas chromatography and high performance liquid chromatography, although these can't provide rapid, sensitive, simple and low-cost on-field detection. Over the last decade more sensitive and specific pesticides detection techniques have been explored, such as fluorescent and amperometric, even if they are not appropriate for routine application, they give a sum parameter of the presence of pesticides without any qualitative or

quantitative information on the individual analyte. In this work, we present a new Raman SERS biosensing system for the qualitative and quantitative detection of pesticides by measuring the Acetylcholinesterase (ACHE) activity. The Raman SERS is not only used for measuring the ACHE activity, but also the direct detection of pesticides individually. Gold nanoparticles (AuNPs) were used as dynamic surface-enhanced Raman spectroscopy (SERS) for sensitive monitoring of ACHE activity in the presence of very low levels of organophosphate and carbamate pesticide. The limit detection of paraoxon and carbaryl were determined at $4*10^{-14}$M and 1nM respectively. These results suggest that this biosensor could be used in the future for the non-selective detection of all ACHE inhibitors at low concentrations.

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Chemical and sensory analysis of chlorine dioxide in drinking water. Part II.
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Drinking water supply companies have traditionally focused their efforts on providing a product with health guarantees, a safe and clean water. Disinfection has been the main purpose of the water treatment. A broad range of disinfectants and technologies are available. Chlorine dioxide has been increasingly used because of its ability to avoid the formation of trihalometans (THMs), the most common and well known disinfection by-products.The origin of the present work was a taste and odour episode which occurred in the water supply system of the city of Barcelona and was attributed to the use of chlorine dioxide for final disinfection in one of the sources. The study had two main objectives: first, develop an analytical method to analyse low levels of chlorine dioxide in presence of chlorine; and second, estimate the organoleptic properties of chlorine dioxide in order to explain the customer complaints. In part I of the project (1) the behaviour of DPD and Lissamine Green B were evaluated. Now, the results of the experiments with amaranth and chlorophenol red (CPR) are presented. It has been demonstrated the amaranth method does not need the addition of glycine, but on the contrary this agent is very useful in the case of the CPR to avoid the chlorine interference. The behaviour of the two methods has been similar. Good calibration curves are obtained although the slope has a poor reproducibility. The validation of both methods with real water samples has showed that the results are acceptable (accuracy error below 20 %) when the concentration of the interfering species, chlorine, is up to 0.8 – 1 mg/L. With respect to the sensory analysis, two odour experiments were performed: discrimination and preference tests between chlorinated vs. dioxichlorinated waters. In part I, some previous results with an untrained panel were presented. Now, additional data with a joint panel including trained tasters are presented. Chlorine dioxide and chlorine can be effectively discriminated. Overall, chlorine dioxide seems to present a stronger odor, although it is not reported as more unpleasant. The results obtained permitted the odour event to be explained: the change of compound for final disinfection was detected by sensitive consumers. (1) R. Devesa et al. Chemical and sensory analysis of chlorine dioxide in drinking water. SETAC Europe 23 rd Meeting, Glasgow, U.K, May 2013. **Keywords**: chlorine dioxide, chlorine, analysis, water.

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EU DEMEAU project: Practical application of in vitro bioassays in water quality assessment
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Through various case studies the usability of bioassays to measure combined effects of emerging and unknown water pollutants were demonstrated within the EU funded project "DEMEAU". The presentation i) explains how (*in vitro*) bioassays can provide cost-effective means for safety evaluations of water samples and investigation of activity profile of single chemicals, ii) introduces a selection criteria system that helps to compile a relevant bioassay panel for the effect-based screening of drinking, surface and waste waters, and iii) summarizes the main results and conclusions of the demonstration studies.**The following studies will be presented: <strong>1. Establishment of bioassay trigger values\n**The need for trigger values is internationally acknowledged and within the project a proposal to derive human health related trigger values has been reported. Ecotoxicological trigger values are being currently developed.
2. Selection criteria to assess the suitability of bioassays\nA recent inter-laboratory study described the application of a broad panel of *in vitro* bioassays to waste, recycled, storm, surface and drinking waters. Each type of water showed a characteristic bioanalytical profile with particular groups of toxicity pathways. Since there are numerous bioassays available for these pathways, we prepared a criteria system to assess the suitability of the respective bioassays and selected the most promising techniques for water quality assessment. **3. Bioscreening of Managed Aquifer Recharge (MAR) samples\n**Various types of MAR sources from two sampling campaigns were subjected to screening in the selected bioassay panel for their toxicity profile and investigate the impact of micropollutants present in these samples. The study revealed the importance of endocrine, oxidative stress and photosynthesis inhibition pathways, and showed differences between the samples collected in two sampling campaigns. **4. Ecotoxicological evaluation of wastewater treatment\n**The efficiency of ozonation and various post-treatments to reduce ecotoxicological effects still occurring in the conventionally (biologically) treated wastewater was assessed in this study. The investigations revealed that the ozone treatment resulted in significantly improved water quality in the majority of bioassays as compared to effects measured in the conventionally treated wastewater. In a few assays, partially higher effects after ozonation occurred, but could be removed by suitable post-treatments.

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Chrysene and dibenz(a,h)anthracene contamination of surface waters: toxicity assessment using water flea (Daphnia magna)
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Water constitutes about 73%of the earth's surface and only 3% of this is freshwater resources. The surface freshwater resource available as liquid is about 0.3%. Several studies and reports across disciplines had established the universal importance of freshwater for human and ecological health. This limited resource is being exploited and degraded daily especially in the developing world. Climate change and it global impacts continue to exert greater pressure on freshwater resources. The need to develop analytical methods, assess pollution levels, evaluate ecotoxicological effects and remediate wastewaters for possible reuse is now a necessity more than ever. This study aimed at developing an analytical method for the qualitative and quantitative determination of chrysene and dibenz(a,h)anthracene in surface waters and to assess possible toxicity effects of these compounds on water flea, *Daphnia magna*. A method with good linearity and precision was developed to measure chrysene and dibenz(a,h)anthracene in water and sediment samples. Acute Daphnia toxicity testing ws carried out to assess potential harm of these contaminants to ecological health. Results of method development, environmental water monitoring and effective concentrations (ECs) are presented.

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Bioavailability and Toxic Response of Bound PAHs in Natural Organic Matters for Oryzias latipes
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This research aims to carry out investigation on bioavailability of PAHs in the present of different NOMs for model animal pelagic Japanese medaka (*Oryzias latipes*). Our previous work has shown that the uptake rate of HOCs in triolein-embedded cellulose acetate membrane (TECAM), a kind of passive sampler, is independent on HA concentrations. Thereby, using TECAMs as a biomimetic tool, combined with biomarkers, the bioavailability of bound PAHs in NOMs to *Oryzias latipes*, as well as the aging effect on PAHs-NOM association and its mechanism was studied. Dissolved humic acid (HA), and four kinds of humins from different sources, were examined in this study as different types of NOMs. The freely dissolved concentrations (C_{free}) of PAHs were estimated by using TECAMs. A bioavailability model as follows was used to describe the bioavailability extent of bound PAHs: $F_{fish} = C_{bioavailable} / (C_{free} + C_{bound}) = (1 + \alpha * K_{OM} * C_{OM}) / (1 + K_{OM} * C_{OM})$, where F_{fish} was used to represent the fraction of bioavailable PAHs, and α is the portion of bioavailable fraction of the bound PAHs. On the basis of the bioavailability model, it was estimated that approximately 20% of HA-bound PAHs contributed to the bioaccumulation in *O. latipes*. However , the bioavailability of PAHs in *O. latipes* was reduced significantly by presence of humins after aging for 32 days. In this condition, the humin-bound PAHs were almost completely unbioavailable, whereas only the freely dissolved fraction can be absorbed by *O. latipes*. The strong affinity between the PAHs and humins after aging blocked the possible bioavailability of bound PAHs, in which the major slow-sorption fraction contributed scarcely to the bioaccumulation. In general, the freely dissolved concentration of PAHs can be used to estimate the bioavailability in most cases, as the NOM-bound fraction of bioavailable PAHs was much smaller.

Multiple stresses in aquatic ecosystems: Assessment of stress response and its consequences in organisms (I)

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Knowledge, Assessment, and Management for AQUAtic Biodiversity and Ecosystem Services aCROSS EU policies
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Aquatic ecosystems are rich in biodiversity and home to a diverse array of species

and habitats, providing numerous economic and societal benefits to Europe. Many of these valuable ecosystems are at risk of being irreversibly damaged by human activities and pressures, including pollution, contamination, invasive species, overfishing and climate change. These pressures threaten the sustainability of these ecosystems, their provision of ecosystem services and ultimately human well-being. AQUACROSS seeks to advance the application of ecosystem-based management for aquatic ecosystems in an effort to support the timely achievement of the EU 2020 Biodiversity Strategy and other international conservation targets. In this regard, AQUACROSS aims to develop and test an assessment framework which considers the full array of interactions, including human activities, within aquatic ecosystems. Existing EU policies have been unable to halt and reverse the trend of declining biodiversity of aquatic ecosystems. The current broad policy landscape such as the Water Framework Directive and Marine Strategy Framework Directive means that sustainable management solutions require coordination and cooperation between different policy areas spanning freshwater, coastal and marine ecosystems, in addition to innovative business solutions and public-private engagement. The AQUACROSS project will support the achievement of EU and international biodiversity targets by delivering a consolidated and coherent outlook on EU policy for aquatic ecosystems; increasing knowledge on biodiversity and drivers of aquatic ecosystem change; supporting the management of Natura 2000 sites and invasive alien species; and testing environmental and business models for the provision of ecosystem services that will contribute to ecosystem protection.

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Assessing dynamics and stability of River Ecosystems under multiple stressors conditions: Iberian rivers as case study

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River ecosystems are complex systems that naturally fluctuate on space and time around a regular pattern or dynamic equilibrium, governed by external environmental conditions. If these are varied up to a threshold, the system may shift towards a new contrasting steady-state or regime. Characterizing the ecosystem stability and predicting the conditions under which such shifts are produced is a challenging task that has become an area of growing interest in the last years [1, 2]. The present work aims at progressing on the understanding of the relationships between the dynamics and stability of Mediterranean River ecosystems and the environmental conditions, taking Iberian Rivers as case study. We also aim at bringing closer modelling and field ecology under a common framework that enables the exploitation and interpretation of field measurements. To do so, we applied a mathematical dynamic model structurally equivalent to the well-known Lotka-Volterra that takes explicitly into account spatial relationships [3]. The model considers both the spatial connection and similarity of biological and other environmental descriptors associated to each river site. Its application provides relevant information regarding questions like the system's stability, transition between states, as well as the interpretation of the longitudinal patterns of the rivers and their evolution according to shifting environmental conditions. By doing so, we will approach modeling and experimental environmental practices under a common framework that facilitates the understanding of ecosystems' behavior as regards to multiple stressors effects. References [1] Rohr RP, Saavedra S, Bascompte J. 2014. On the structural stability of mutualistic systems. Science 345: 416-425 [2] Scheffer M, Carpenter SR, Lenton TM, Bascompte J, Brock W, Dakos V, van de Koppel J, van de Leemput IA, Levin SA, van Nes EH, Pascual M, Vandermeer J. 2012. Anticipating critical transitions. Science 338: 344-348 [3] Dakos V, van Nes EH, Donangelo R, Fort H, Scheffer M. 2010. Spatial correlation as leading indicator of catastrophic shifts. Theor. Ecol. 3: 163-174 *Acknowledgement* - This work is supported by the European Communities 7th Framework Programme funding under Grant Agreement No. 603629-ENV-2013-6.2.1-Globaqua

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Natural variability of biochemical biomarkers in the macro-zoobenthos: dependence on life stage and environmental factors

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Biomarkers have been widely implied in ecotoxicology as indicators of exposure to toxicants. However, the usefulness of biomarkers in ecotoxicology and, in particular, their ability to provide unambiguous and ecologically relevant information on exposure to or effects of stress factors remains controversial. One of the major problems for the use of biomarkers in ecotoxicology is understanding if the measured responses are determined by the effects of stress factors or lie within the natural variability range produced by the effect of environmental parameters. In a previous work, the natural variability of enzymatic levels, measured in some freshwater invertebrate taxa sampled in pristine rivers, was proved to be relevant across both space and time. In this work, the experimental design was improved by considering different life stages of the selected taxa and by including the measure of additional environmental parameters. The work aimed at evaluating the variability of some enzymatic biomarkers measured in freshwater benthic invertebrates collected in pristine alpine streams in order to limit any potential anthropic influence. The experimental design considered: sampling sites in two different rivers; eight sampling dates covering the whole seasonal cycle; four taxonomic groups (Perlidae, Baethidae, Heptageniidae, Hydropsychidae) present in almost all samples; three to four different life stages considered for each taxonomic group; four enzymes: Acetylcholinesterase, Glutathione-S-transferase, Alkaline phosphatase, and Catalase. Biomarkers levels were related to several environmental parameters (temperature, pH, conductivity, oxygen level, nitrogen and phosphate concentrations, metals and metalloids concentrations), to verify any kind of dependence. Data were elaborated using multivariate statistical methods. Natural variability of enzymatic levels was found to be relevant across both space and time. The results of this work proved that great care should be paid when interpreting experiments in which biomarkers levels are measured and compared among sites or dates; further research is needed to understand how the natural variability of biomarkers could be accounted for and managed in ecotoxicological studies.

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How do anthropogenic pollutants affect the genetic structure of a model invertebrate freshwater population?

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Organisms are rarely exposed to only one single anthropogenic stressor in the environment, but rather to multiple human-derived threats acting simultaneously. Environmental pollution can modify genetic population structure via ecological bottlenecking, adaptation of species to contaminants, altered gene flow, or increasing mutation rate. Organic micropollutants such as pesticides, biocides, pharmaceuticals, personal-care products, or industrial chemicals are ubiquitous in the aquatic environment and their effects are considered a relatively new and emerging anthropogenic pressure over evolutionary processes. Especially potential effects of pollutants on genetic population structure may be more disruptive regarding ecosystem functioning than individual-level effects. Despite the bunch investigations on genetic variation in wildlife, our understanding about the individual stressor effects on genetic variation is still limited. Recently, there has been an increased interest in integrating environmental chemistry and evolutionary ecotoxicology approaches into the assessment of direct and indirect effects of anthropogenic pressures on populations. To address these challenges the genetic structure of a shredder invertebrate, *Gammarus pulex*, was examined using evolutionary ecotoxicology and body burden approaches. Significant genetic depression was detected and remarkable presence of private/rare alleles occured after first WWTP effuents. These effects occured together with a drastic increase in pollutants together indicating their mutagenic activity. Thus, it is highly probable that mutagenic chemicals emmited by the WWTP have a significant impact on genetic variation of gammarids. Conversely, a slight though significant increase in genetic diversity was detected in a short stretch of the river (~four kilometers) marked by rainwater drainage, the second wastewater treatment plant effluent and a small backflow level upstream the weir. These sampling sites showed a strong significant correlation in RDA analysis performed in order to assess the influence of man-made stressors on genetic diversity and structure. This study provides evidence of direct and indirect effects of anthropogenic pollutants on genetic variation in an invertebrate freshwater population. Clear signal of pollutants-induced genetic changes were determined in the Holtemme River.

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Influence of chemical and non-chemical stressors on the macroinvertebrate community composition of the Danube river

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A key challenge for the evaluation of their impacts of chemical pollution in aquatic ecosystems has been to disentangle their isolated toxic effects caused by water contaminants from those originated by other sources of natural or anthropogenic stress. In this study we developed a method for the evaluation of single and combined effects of chemical pollution on aquatic communities of large rivers. The method was based on the combination of univariate and multivariate statistical techniques including GLMs, PCA, RDA and Variation Partitioning. The method was applied to the database obtained as part of the environmental monitoring campaign performed during 2013 in the Danube river and used in the SOLUTIONS project. The database contained information on the macroinvertebrate community from 55 sampling sites and measurements of more than 300 environmental parameters including hydromorphological conditions, physical river alterations, and basic water quality parameters and contaminants (i.e., pharmaceuticals, pesticides, metals, industrial pollutants, etc). The results of this study indicate that the variation in the invertebrate community is principally explained by the combination of hydromorphological conditions and water contaminants. The most important contaminant groups were pharmaceuticals and basic water quality parameters (e.g. nutrients, DO). Furthermore, a trait-based analysis was performed that indicates correlations between the presence of contaminants and functional characteristics of the invertebrate communities. The results of this study indicate main contaminants, species and biological traits that should be further monitored for the evaluation of the ecological status of the Danube river and serves as reference to other river basins in Europe.

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Freshwater bivalves detoxification of microcystin-LR and Roundup Flash® differ with species: implications for adaptation?
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Species composition in anthropogenic-impacted environments may depend on the capacity of single species to realise their niches within the pollution scenario. Adaptation can be facilitated via different mechanisms, one of which is the efficiency of detoxification. Many indigenous freshwater mussels species are on a decline, whereas invasive ones proliferate in the same pollution scenario, e.g. in water bodies adjacent to farmlands receiving both, pesticides and fertilizers, which mussels would accumulate by feeding via filtration. One of the most used herbicides worldwide is glyphosate, for its seemingly specific inhibition of the amino acid synthesis in plants through the shikimic acid pathway. Evidence about its harmful effects in non-target organisms is rising, in particular for the commercial formulations. Fertilizers stimulate cyanobacterial blooms, which harm organisms via toxic metabolites. Of these cyanobacterial toxins, microcystin-LR is frequent; it inactivates protein phosphatase of type 1 and 2A, causing cellular malfunctions until death of the organism. Within exposure concentration and duration limits, microcystins can be detoxified via conjugation to glutathione by the glutathione S-transferase (GST) enzymes. This study compares biotransformation of microcystin-LR and the herbicide Roundup Flash® (containing Glyphosate as a.i.) and energy allocated to that in an invasive (*Dreissena polymorpha*) and indigenous mussel species (*U. tumidus* and *U. pictorum*). The invasive *D. polymorpha* detoxified microcystin-LR up to 50 μgL^{-1}*via* the sGST but *U. tumidus'* sGST were rather inhibited in the 7 day exposure. Moreover, GST was hampered after 7 days in both indigenous species by Roundup Flash® and could not increase in the mixed exposure. Thus the detoxification capacity of microcystin-LR was limited in the mixed exposure, which lead to enhanced accumulation of the cyanobacterial toxin. Detoxification and other mending processes consumed energy, however the species drew from differen resources: *D. polymorpha* and *U. tumidus* first used glycogen, and after 7 days of exposure *U. tumidus* but not *D. plymopha* consumed lipids. Contrastingly, *U. pictorum* needed the lipids and thus more energy right from the start. Again, *D. polymorpha* seems to combat the environmental stress with less effort. Different life trait consequences may occur via the energy spent, thus potentially lacking for growth and reproduction, leading to consequences on the population level.

Identification and prioritisation of hazardous pollutants in the aquatic environment - the role of effect-directed analysis, monitoring and modelling (I)

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A conceptual framework for a solutions-focused management of chemicals
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Risk assessment and prioritisation procedures have in common that they are based on extensive research and evaluation and focus on one chemical substance at a time, in contrast to real world contamination comprising thousands of chemicals. Challenges remain to link the occurrence of chemicals to the status of waters, to identify major chemical stressors including mixtures, and to find solutions for abatement. The project SOLUTIONS will address these challenges. To manage the SOLUTIONS approach, a conceptual framework was developed with four main entry points: **Chemicals, Environment, Abatement and Society** based on needsexpressed by end-users. The entry points are linked by four topics which describe the scientific challenges that will be addressed: Under the activity ***Identify and prioritize hazardous chemicals on different scales***, the aim is to provide a hypothesis on causes and candidate chemicals starting with existing monitoring data and applying innovative approaches for improved monitoring such as target and non-target screening, bioanalytical tools and effect-directed analysis – EDA. Modelling using an integrated 'model train' to link emissions to impacts provides a useful tool to fill data gaps and to extrapolate. The activity ***Selection of abatement options*** is focused on developing guidance on selection of and placement of abatement options. The topic ***Decision support for management of chemical pollution*** is aimed at providing guidance on existing and future policy frameworks. Potentials for synergies will be assessed and gaps in current policies identified e.g. for groups of chemicals or specific sectors in society not or poorly covered by existing legislation. Under the activity ***Predict and prioritize future risks*** the approach is to assess future emissions based on an evaluation of relevant societal scenarios (economy, demography, industry, consumption, energy, agriculture)and how these trends and scenarios may affect use and emissions of chemicals. SOLUTIONS will deliver a number of important products which can be used for the implementation of a sustainable use of chemicals in Europe. The information and experiences will provide the basis for introducing an innovative solutions-focused approach in risk assessment for emerging substances.
Acknowledgements The SOLUTIONS is a EU FP7 project, grant agreement 603437.

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20-years record of emerging and priority pollutants in salmon tissues from southwestern France
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Coastal rivers are subject to human pressures often combined with the emission of potentially hazardous organic and inorganic contaminants. In addition, some pollutants in previous decades have accumulated in sediment and can be leached into the water column under certain conditions such as floods. Without ending up at alarming levels in terms of toxicity, many contaminants can impact aquatic wildlife. Traditionally, the most studied pollutants correspond to the priority substances listed in the Framework Directive (WFD) such as pesticides, metals, organometals, PAHs and PCBs. There is however a growing awareness of the occurrence of emerging pollutants for which the elimination processes in wastewater treatment plants are not yet adapted. Among them, the musk compounds for which there is currently no environmental quality standards set by the WFD. Synthetic musks are primarily components of personal care products. Because of their ubiquity and lipophilic properties, some of them are found in aquatic organisms, but few data still exist. The monitoring of priority substances and other pollutants, including new emerging substances, requires the implementation of temporal and spatial trend monitoring programs. The use of integrative matrices (biota and sediments) is strongly recommended to achieve such objectives. Fish are ideal for contaminant monitoring as they accumulate pollutants both from the surrounding water and their prey through processes bioaccumulation and biomagnification. This study aims to investigate the occurrence and concentrations of emerging and priority pollutants in various organs (liver, muscles and gonads) in Atlantic salmon (Salmo salar). We investigate the temporal variations of several chemicals (including organochlorine pesticides, HAPs, PCBs, metals and organometals, synthetic musks) and their metabolisation in salmons caught in the Nivelle river (Southwestern France). Several biophysical parameters are also monitor for each individuals (weight, length, sex, age, percent lipid) as well as ecological parameters (C and N isotopic signatures, otolith chemical signatures, scales fingerprinting) over a 20 years period. This monitoring permits to define status and trends for these contaminants, but this multi-contaminant approach gives also inputs to raise important questions for both science and policy such as where does the contamination come from and how can the situation be resolved?

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Impact of untreated wastewater on a major European river evaluated with a combination of in vitro bioassays and chemical analysis
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The pollution of river ecosystems by complex mixtures of micropollutants, such as pesticides, pharmaceuticals and industrial chemicals is of concern for ecosystem health and for use as drinking water source. Untreated wastewater is discharged into the River Danube in Novi Sad, Serbia, which triggered the

investigation of the load of micropollutants by chemical and bioanalytical analysis applied to three on-site large volume solid phase extracted water samples from the Danube upstream and downstream of a wastewater discharge point. A battery of *in vitro* cell-based bioassays covering important steps of the cellular toxicity pathway revealed prominent effects on the activation of metabolism (arylhydrocarbon receptor AhR, peroxisome proliferator activated receptor gamma PPARγ), endocrine disruption (estrogen receptor ER, androgen receptor AR) and adaptive stress responses (oxidative stress, inflammation). Of the analyzed 261 compounds 112 were detected at least in one sample. Both chemical and biological analysis revealed the same pattern. The site upstream and 7 km downstream of the discharge had a similar burden of chemicals and effects whereas directly downstream of the wastewater discharge effects and micropollutants' concentrations were increased, particularly those typically associated with wastewater. The detected chemicals could explain 28% of AhR activation, 62% of AR inhibition and 24% of glucocorticogenic inhibition in the water extract directly downstream of the wastewater discharge. Thus the direct wastewater discharge increased the micropollutant load, but the large volume of the receiving stream Danube diluted them within a few kilometers to a status similar to upstream of the discharge. Keywords: bioassay; chemical analysis; Danube; Serbia

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Detection of physiological activities of pharmaceuticals in river water: Suggestions to prioritization of pharmaceuticals in research involving environmental monitoring and toxicity testing

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Over recent years, growing numbers of human pharmaceuticals have been detected in effluents of wastewater treatment plants (WWTPs) and river water. Although these are generally found at very low levels (e.g., ng/L to µg/L) in these waters, concern about their potential risks to aquatic species has been raised because they are designed to be biologically active. To determine whether pharmaceuticals in aquatic environments pose risks to aquatic organisms, we must know the extent to which such organisms may be exposed to those pharmaceuticals as determined by the pharmaceuticals' respective modes of action. G protein-coupled receptors (GPCRs) are the largest group of these cell surface receptors in eukaryotes, and participate in various physiological and pathophysiological processes. It is estimated that nearly half of all marketed pharmaceuticals act by binding to GPCRs: for example, antihypertensives, antipsychotics, antidepressants, antiallergenics, and antiasthmatics. In 2012, Inoue et al. developed an *in vitro* transforming growth factor-α (TGFα) shedding assay, in which GPCR activation is measured as ectodomain shedding of a membrane-bound preform of alkaline phosphatase-tagged TGFα and its release into conditioned medium. The TGFα shedding assay can detect not only activation but also inhibition of GPCRs, and is a very simple and rapid tool. In this study, we measured the antagonistic activities of GPCR-acting pharmaceuticals in WWTP effluents, and upstream and downstream of the WWTP outfall in Japan using the TGFα shedding assay. We selected receptors for angiotensin (AT1), dopamine (D2), acetylcholine (M1), histamine (H1), and adrenergic family member (β1). Activities detected in waters were quantified as antagonist equivalent quantities (EQs). Antagonistic activity against AT1, D2, M1,H1, and β1 receptor were detected in the final effluent of WWTP A through two years. Particulaly, antagonisitic activity agaainst AT1 and H1 receptor were strong, up to several µg/L as olmesartan-EQ (µg-OM/L) or diohenhydramine-EQ (µg-DIP/L), respectively. Antagonistic activities against AT1, H1, and M1 receptor in downstream of river were higher than those in upstream due to the effluent from the WWTP A. Based on thse results, we propose that pharmaceuticals antagonistic to AT1, D2, H1, M1 and β1 receptors should be prioritized in future environmental monitoring and toxicity testing.

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Enhanced Environmental Non-target Screening: Connecting Mass Spectrometry, Background Knowledge, Data Sources and Predictions

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Non-target screening using high resolution mass spectrometry (HR-MS) is essential to help prioritize and identify the tens of thousands of unknown chemicals detected in complex environmental mixtures, but is also time-consuming and requires compilation of information from a plethora of sources. A high-throughput, consolidated non-target identification workflow for HR-MS was developed within the European project SOLUTIONS. The *in silico* fragmenter MetFrag was expanded to include retention time (RT), reference and patent information, spectral similarity, suspect list screening, element and/or substructure selection and exclusion as well as user-defined scores [1,2]. References and patents provide vital clues for high-use substances, while suspect screening allows candidate retrieval from large compound databases (several million entries) combined with tagging entries in various suspect lists (e.g. STOFF-IDENT [3], NORMAN Suspects[4]). User-defined scores allow e.g. the inclusion of per-substance toxicity predictions relevant for effect-directed analysis (EDA). MetFrag2.2 was evaluated on 1308 merged spectra of 975 environmental reference standards from MassBank, with 71 % ranked correctly in first place using fragmentation, RT and reference/patent information. The workflow in R covers peak-picking to identification with enviPick, enviMass, enviPat, nontarget, RMassBank and ReSOLUTION. A graphical user interface, enviPy, is also available. The workflow was tested on upstream, downstream and effluent samples from three locations in the SOLUTIONS Rhine case study. Masses (159 positive, 137 negative mode) were prioritized for identification using peak picking, componentization (isotope, adduct information) and pattern analysis. Transformation products of common surfactants were clearly formed during treatment and present downstream. Downstream of Muri WWTP, 110 high intensity non-targets were investigated, with 83 suspects from STOFF-IDENT (7602 substances, including REACH) among 72,331 total candidates retrieved from ChemSpider. The top matches for 54 masses are highly promising; confirmation efforts are underway. The workflow greatly expedites high-throughput non-target screening and has huge potential to assist prioritization and EDA. [1] Ruttkies *et al* 2015, J. Cheminformatics, under review [2] http://c-ruttkies.github.io/MetFrag/ [3] http://bb-x-stoffident.hswt.de/ [4] http://www.norman-network.com/?q=node/236

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Revisiting the Mutagenicity in River Rhine: Identification of Mutagenic Aromatic Amines

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Since the 1970s mutagenic activity was observed in many samples from the lower part of River Rhine and although the mutagenicity of the river decreased by time, the activity is still detectable. The mutagenicity increases significantly with metabolic activation in AMES test and comet assays pointing at the contribution of mutagenic aromatic amines. However to best knowledge of the authors, the responsible compounds still remain unidentified. Thus, the aim of this study is to detect and identify the cause of mutagenicity in River Rhine using effect-directed analysis. Samples were taken at Lobith, Netherlands by an on-site large volume SPE machine using a polymeric sorbent. The extract was fractionated using a semi-preparative C18 column and two-minute fractions were collected. Ames fluctuation assay (Ames II test) was used to assess the mutagenicity with tester strain TA98 and strain YG 1024, which has an increased sensitivity for aromatic amines with metabolic activation. The raw extract showed a significant mutagenicity in the presence of S9 with TA98 and YG1024 at a relative enrichment factor of 250. 10 active fractions were detected with TA98 strains and 5 additional active fractions were observed with YG 1024 strains in the presence of S9. The number of revertants increased significantly with YG1024 strains confirming the contribution of mutagenic aromatic amines. The active fractions were analyzed using an ion trap-Orbitrap hybrid instrument (LTQ Orbitrap XL, Thermo Scientific) in ESI positive ion mode by a previously developed pre-column diagnostic derivatization method to selectively label amines. The overview of the first data evaluation reveals the abundancy of compounds with amino groups in the river. Two compounds, namely, Sotalol a commonly used betablocker and Sulfapyridine an antibacterial agent were confirmed by reference standards. Nortilidine and Bisnortilidine, two metabolites of the opioid painkiller Tilidine were found as the only Metfrag candidates and the parent compound, Tilidine, was confirmed with the MassBank spectra match. Moreover, 5-Methyl-1H-benzotriazole, Lamotrigine, p-toluidine and 1H-benzotriazole(BTA) were also confirmed by MassBank spectra matches. In addition to these compounds, MetFrag candidates point out aminoquinilones and other imidazole containing compounds which are currently being evaluated.

Standards - an essential link between environmental science and regulation

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A regulatory view on the application of standardised tests

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Cefas acts as the UK Government regulators or advisors to the regulators in a number of Marine fields, and also runs a separate ecotoxicology facility which enables us to view the use of standardised testing from more than one perspective. We discuss how the availability of standardised tests affects the regulator and those involved in submitting reports and testing. A case study is examined where the use of standardised tests allowed the regulator to re-assess data from previous submissions Aspects of standardised tests that allow regulations to operate effectively are discussed. Is it critical for the standardised test to be chosen carefully to represent the area of application. A scientific view and a regulatory view are presented. We present a view of how regulatory roles would be affected in the absence of standardised tests We conclude that standardised testing provides a solid basis for regulatory decisions, though should not be the only information permitted OR requested.

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Standards in Environmental Toxicology - Strengths and Weaknesses - Practicality versus Complexity
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The strength of Standards in Ecotoxicology is that the users not only know exactly - but also have to abide by - all the detailed descriptions. The Standards prescribed by national and international organizations for toxicity testing unfortunately often differ - sometimes even to a substantial extent - on many aspects. These differences clearly bear an influence on the outcome of the assays. Several examples of the differences of prescribed standard toxicity test methods will be highlighted with regard to e.g. selection of test species, effect criteria, exposure time, number of test organisms and replicates, validity criteria and data analysis. A major drawback of many Standards is that only little attention has been paid to their practicality/complexity aspects. It is often argued that for the evaluation of the hazards of chemicals - as is e.g. the case for REACH dossiers on chemicals - these "inherent" practicality/complexity aspects have to be coped with. Standard toxicity tests are, however, also needed for application in a regulatory framework for routine toxicity assessment of polluted environments, and practicality and cost problems are in this regard a major point. Independently of Standards, scientists have addressed the former drawbacks by developing "small-scale" toxicity test methods, and the name "microbiotests" was coined in this regard in 1991 by Dr. C. Blaise. Microbiotests must be inexpensive or cost-efficient, generally not labor intensive, have high sample throughput potential, be based on cultures which are easily maintained or maintenance free, require modest laboratory and incubation space, low sample volumes and be precise and reproducible. At the turn of the century, the very first simple, rapid and cost-effective microbiotest (the "bacterial luminescence inhibition assay") was proposed and accepted by the ISO and is now in use worldwide. Extensive research on "stock culture/maintenance free" microbiotests has since then been performed at the Ghent University in Belgium, with test species belonging to different phylogenetic groups (algae, protozoans, rotifers, crustaceans, and plants). Validated by extensive International Interlaboratory Comparisons, several of these microbiotests have been accepted by the ISO and because of their "simplicity and practicality" and their availability in kits, these microbiotests are to date also applied worldwide for research and routine toxicity monitoring.

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Implementation of the TRIAD approach for the ecological risk assessment of an old lead mine.
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Site specific ecological risk assessment of soil contamination goes with intrinsic difficulties. Obvious ones are heterogeneity of soil properties, landscape, local communities, diversity and ecosystem structure, and contamination nature and pressure. In this context, the International Standard ISO/DIS 19204 "Soil quality – Procedure for site specific ecological risk assessment of soil contamination (Soil quality TRIAD approach)" describes in a general way the application of three combined lines of evidence (chemistry, ecotoxicology and ecology). The TRIAD includes different tiers in which each consecutive tier is increasingly fine-tuned according to the site-specific situation. Data collected on an open pit mine operated during 60 years and which the activities stopped about 100 years ago as been used to evaluate the ease and efficiency in using the different tiers of TRIAD. The pollution pressure still present in the mine is of metallic origin (mainly Pb, Cd, Zn) and 5 areas with different levels of contamination, vegetation and quantites of organic matter in soil were chosen. It was possible to apply the tier one tools on every selected locations. The combined use of chemical concentration in soil (Chemistry), plant screening test (Toxicology) and simple vegetation survey (Ecology) were sufficient to asses the risk on some of the areas. In consequence, some of the expensive and time consuming data collected in these locations were not needed to assess the risk. On other locations, uncertainties were still too great and the implementation of higher tiers was needed. The focus during this applied case was on the outcome of the TRIAD method in order to obtain practicable results according to the complexity of the study. It reveals that for some locations, the use of TRIAD method can save financial and time ressources. The use of the highest tier on particularly complex zones will need additionnal datas, future investigations are planned for the year 2016.

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Ecotoxicological assessment of corrosion protection products used on hydraulic steel structures
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Corrosion protection is applied to extend the lifetime and function of steel components. In our study we conducted market research to establish which corrosion protection products are used in Switzerland. It appeared that epoxy resins have ca. 50% market share. Subsequently, we ran laboratory leaching tests with four products and determined the ecotoxicity of leachates using a suite of bioassays. The bioassays we ran were mainly (draft) ISO standards and one non-standard bioassay (the combined algae test) which has been applied in Switzerland in a regulatory context. However, currently no uniform or internationally adopted scheme is available to evaluate results from bioassays run on leachates from construction materials. For this reason we used a German scheme developed by DIBt (German Centre of Competence for Construction) to evaluate our bioassay results. In future, a scheme may become available through CEN (European Committee for Standardisation). CEN is currently preparing a report that will highlight the potential of bioassays for the evaluation of leachates from construction materials. Four types of epoxy resin based products were obtained and prepared according to instructions of the producers. Resins were directly applied onto glass plates without any primer. Two leaching experiments were performed as 7-day horizontal shaking tests using a volume to surface ratio of 10 L/m^2. Toxicity was observed in all tests and particularly two products showed elevated toxicity. For Product 1, a >1000-fold dilution was required to reduce bacterial bioluminescence inhibition under 20% (a threshold specified in the DIBt scheme). Samples from Product 3 induced large effects in several receptor activation assays. Bisphenol A measurements in these samples clearly linked this compound to the observed endocrine effects. Although worst case scenarios were tested, i.e. short hardening times and low volume to surface ratios, the observed effects often require high leachate dilutions to reduce effect-levels below 20%. The DIBt evaluation scheme proved useful for the ecotoxicological evaluation of the leachates. However, a uniform and internationally standardised and adopted evaluation scheme – covering an extended effect panel – is desirable.
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Advancing the use of passive sampling in risk assessment and management of contaminated sediments: Results of an international passive sampling ring test
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It is widely-accepted that it is not the total (solvent-extractable), but only the bioavailable fraction of hydrophobic organic contaminants in sediments that is available for uptake in organisms and responsible for any subsequent effects. In view of proper risk assessment and management of contaminated sediments, several methods for assessing bioavailability have been developed and are being used; however, primarily within the scientific community. Among these methods, passive sampling (i.e., determining freely dissolved concentrations; C_{free}) represents the most widely-used and well-characterized approach. In some regulatory communities, there is aversion to implementing these methods in actual risk assessments, as there is no consensus among scientists on which bioavailability assessment or passive sampling approach is best to use and the variability associated with these methods remains largely unknown. Therefore, the objectives of this international ring test study were (1) to map the state of the art in determining C_{free} with passive sampling (i.e., what is the intra/inter-method/lab variability); (2) to identify the sources of variability by means of dedicated, tiered experiments (including standardizing methods); (3) to provide recommendations and practical guidance (standard protocols); and (4) to increase confidence in the use of passive sampling and advance its use outside of the scientific domain. The ring test was performed by a consortium of 11 labs and included experiments with 14 passive sampler types on three sediments and 25 chemicals (i.e., PAHs and

PCBs). The results demonstrated that standardizing methods significantly decreased the overall inter-lab variability. The resulting variability however still largely exceeded the intra-lab/inter-method variability. By performing all analyses in one laboratory, this difference in part could be explained by the substantial variability introduced through analytical chemistry (i.e., identification, integration, calibration of target chemicals). Overall, the findings of the ring test suggest that passive sampling is fit for implementation in risk assessment and the management of contaminated sediments when following standard protocols.

A focus on research and education tools in environmental toxicology and chemistry

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A coordinated set of ecosystem research platforms open to international research in ecotoxicology, AnaEE-France
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Human activities have altered continental ecosystems worldwide and generated a major environmental crisis. To truly develop integrative ecosystem biology and to assess the consequences of various forcing factors, such as pollutions, we therefore need new approaches and tools that bridge the traditional gap between life and environmental sciences. To deal with that challenge, the infrastructure for Analysis and Experimentation on Ecosystems (AnaEE-France) is an integrated network of the major French experimental, analytical and modeling platforms dedicated to the biological study of continental ecosystems, both aquatic and terrestrial. This infrastructure aims at understanding and predicting ecosystem dynamics under global change. It comprises five complementary nodes offering access to the best experimental facilities and associated biological resources and data: Ecotrons, semi-natural experimental platforms to manipulate terrestrial and aquatic ecosystems, *in natura* sites equipped for large scale and long-term experiments. AnaEE-France also provides shared instruments and analytical platforms dedicated to environmental (micro)-biology. Finally, AnaEE-France provides users with data bases and modeling tools designed to represent ecosystem dynamics and to go further in coupling ecological, agronomical and evolutionary approaches. AnaEE-France offers adequate services to tackle the new challenges of research in ecotoxicology, positioning its various types of platforms in an ecologically advanced ecotoxicology approach. AnaEE-France is a leading international infrastructure and it is pioneering the construction of a European AnaEE infrastructure in the field of ecosystem research. This infrastructure is open to the international community of scientists in the field of continental ecotoxicology.

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MOSAIC: a web interface with modelling and statistical tools for ecotoxicology
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MOSAIC stands for "*MOdeling and StAtistical tools for ecotoxicology*". In ecotoxicology, bioassays are standardly conducted in order to measure acute or chronic effects of potentially toxic substances on reproduction, growth and/or survival of living animals. MOSAIC is a user-friendly web interface dedicated to the mathematical and statistical modelling of such standard bioassay data. Its simple use makes MOSAIC a turnkey decision-making tool for ecotoxicologists and regulators. Without wasting time on extensive mathematical and statistical technicalities, users are given advanced and innovative methods for a valuable quantitative environmental risk assessment. MOSAIC is available at http://pbil.univ-lyon1.fr/software/mosaic/. Today, MOSAIC offers three operational tools : (i) **MOSAIC_SSD**, a tool dedicated to the species sensitivity distribution (SSD) approach aiming at defining safe levels for toxic compounds in an ecosystem through the calculation of the so-called hazardous concentration for *p*% of the species (*HCp*), even when the toxicity values are censored; (ii) **MOSAIC_repro**, which provides users with a complete statistical analysis of bioassay reproduction data simultaneously accounting for mortality all along the bioassay. Concentration-response models are fitted within a Bayesian framework to provide ECx estimates; (iii) **MOSAIC_surv**, which provides users with a complete statistical analysis of bioassay survival data. A log-logistic model is fitted within a Bayesian framework to provided LCx estimates. This presentation is an overview of MOSAIC features based on illustrative examples as provided within the web interface.

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ImpactE: an emerging innovation center in environmental performance of processes, products and ecosystems
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The innovation center ImpactE, created in 2015, aims at transferring scientific knowledge and technical skills in ecology, ecotoxicology, microbiology, bio-geochemistry and physico-chemistry, from the laboratory to the industry. The targeted partners are manufacturing industries concerned with the environmental impact of their activities, environmental engineering companies, and companies involved in sustainability development. The activity is presently focused on assisting small and medium size companies to improve their eco-efficiency, and thus to increase their economic health. Therefore we raised co-funding from Municipal and Regional authorities, the French State and Europe. ImpactE offers : Studies to improve environmental performance of manufactured products, taking into account ecotoxicity risk assessment, according to the physical and chemical properties of the products, Studies to reduce the environmental impact of manufacturing, according to sustainability development concepts : waste and sewage management, monitoring of contaminated sites, remediation technologies, Training courses for companies personal in ecology, ecotoxicology, risk assessment, … The ImpactE team provides high level scientific skills acquired by a long time experience in the multi-disciplinary research areas. We also can help our partners to perform funding arrangement of collaborative projects. Keywords : environmental risk assessment ; environmental performance ; research and education tools ; innovation

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The NORMAN network's special view on prioritisation of biocides as emerging contaminants
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NORMAN promotes the use of innovative monitoring and assessment tools for identifying the substances of emerging concern most in need of future regulation. The network maintains various databases (e.g. EMPODAT) and has developed a prioritisation scheme specifically designed to deal with "problematic" substances for which knowledge gaps are identified. These tools have been significantly improved in recent years (expansion of EMPODAT database from 1 million to more than 6 million records; a new "ecotox" module to allow systematic collection of ecotoxicity test data from online databases worldwide, plus existing regulatory EQS/PNEC values). The NORMAN list of "frequently discussed" emerging substances contains 862 compounds: among them, 253 are "new" substances which have been added to the previous list from 2013, whereas 100 substances are now labelled as "former NORMAN" emerging substances. As regards biocides, the list contains 151 active substances of emerging concern that are still in use, under review or formerly used and 12 compounds (e.g., cybutryne, cypermetryne, dichlorvos, etc.) that are still listed for data collection but labelled as "former NORMAN" compounds. The NORMAN prioritisation scheme helps to identify some compounds which evidently need control / mitigation measures (e.g., deltamethrine, terbutryn, imidaclopride, carbendazim, triclosan). Moreover, it is possible to cite substances for which additional monitoring data would be needed,such as e.g., fenoxycarb and tolylfluanid with a potential risk of exceedance of the PNEC. Cyfluthrin and permethrin were identified as substances for which analytical performance should be improved (target: achieve LOQ < PNEC) and N,N-diethyltoluamide and propiconazole appear as substances already sufficiently monitored and for which no evidence of risk was identified. Biocides are active substances emitted into our environment which are definitely to be regarded as substances of emerging concern. EMPODAT confirms that biocides are still insufficiently covered in monitoring programmes: data are available for 70% of the compounds that are also used as plant protection products, but only 15% of the compounds used solely as biocides have monitoring data in the database. Access to the latest information on emerging pollutants, with an overview of benchmark values on their occurrence across Europe would certainly be of a major importance for risk assessors.

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Master Ecophysiology Ecotoxicology Université Pierre et Marie Curie - Sorbonne Universités
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The EPET specialty is a new specialty at the University Pierre et Marie Curie (UPMC) that is part of the important scientific restructuring implemented in recent years at UPMC around environmental sciences. This specialty complete the other master programs by developing more particularly environmental issues at the organism levels. Lessons taught by various specialists have the objectives to train students to: - The complexity of the relationships between organisms and their environment - The response of microorganisms, animals and plants to changes in their environment (land and water), and impact on ecosystems. - The acclimation mechanisms and detoxification / resistance organizations: plastic phenotype expression, epigenetic ... - The different approaches used in an environmental context and laboratory molecular biology, biochemistry, physiology, ethology, ... - Abiotic stresses (temperature, light, salinity, pollutants ...) and biotic (food availability, parasitism, symbiosis, ...) in a context of human impact and climate change. - The importance of the entire organization in solving environmental, industrial and management / protection of species and ecosystems in the framework of sustainable development. This training involves many researchers developing ecophysiology and / or ecotoxicology research in different sites of UPMC ... ... on different models (micro-organisms, plants and animals) living in aquatic and terrestrial environments. ... with the involment of various organizations and companies working in the field

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PROFETIA: Open and Online Training Program in Aquatic Ecotoxicology
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ECOBIM, the international research network in aquatic ecotoxicology was initiated in 2001 by researchers from Quebec (Canada) and France, who shared projects research in aquatic ecotoxicology. While several researchers from countries in Europe, Africa and the Americas participated in the annual meetings, it seemed important to share their teachings to promote transfer of knowledge, harmonizing approaches and methods, and to supplement courses with notions sometimes absent. It was proposed to create a training program that understand different credited modules consisting of digital educational resources available online, as well as methodological harmonization workshops offered in presence at the annual meetings of ÉcoBim. This training program that would be offered to the Master and doctoral level, and initial training or continuous would be the scientific know-how of the ÉcoBIM researchers. This strategic partnership in the field teaching involves the design of educational tools in aquatic ecotoxicology. The PROFECIA project, its objectives and its implementation will be presented.

Advances in Social Life Cycle Assessment and Life Cycle-based Sustainability Assessment

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Data quality management in Social LCA studies
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Typically, many different information sources are consulted for a social LCA case study, from surveys to company information to public sources of various origins. As social data can change rather quickly, and since several sources can contribute, following a triangulation approach, to a final data point used in a study, an assessment and effective handling of the data quality of social information is important for social LCA. This holds both for social LCA case studies and for generic databases used for them. Understanding the data quality is important especially if the study is used for decision support. An approach for addressing data quality for social LCA studies is presented. The approach uses a specific pedigree matrix to evaluate the quality by 5 different indicators, following evaluation scores from 1 to 5. This matrix is implemented in an LCA software (openLCA), and it is also supported by the social LCA database PSILCA. In a case study on latex products, it is shown how both together support the management of data quality for social LCA in decision support, to detect e.g. whether hot spots appear in the case study to some extent because of weak data quality, or whether hot spots call for immediate action because they are based on reliable information. And, of course, also vice versa, there may be cases where hot spots are not visible because the available information is weak, hiding hot spots.

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A methodology for social impact assessment of nano-enabled products
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It is widely agreed that the entire Life Cycle of nano-enabled products is the appropriate unit of analysis to consider its sustainability aspects, including its social impacts. However, there is limited information on which social impacts are relevant for nano-enabled products, and a methodology to monitor them is lacking. In the EU SUN (Sustainable Nanotechnology) project a quantitative methodology based on Social Life Cycle Assessment (s-LCA) and Multi-Criteria Decision Analysis (MCDA) is proposed to assess the social impacts of nano-enabled products through their Life Cycle. The s-LCA conceptual scheme (i.e. impacts and indicators for different stakeholders) is developed through an appraisal of literature on social impacts of products and Ethical Legal and Social Impacts of nanotechnology, and comprises of nine impacts each for workers, consumers and community and six impacts for value chain actors. Out of them, five indicators associated with impacts of nano-enabled products, with two impacts in Worker category (Professional training and Non-fatal accidents) and three impacts in Community category (Education, Employment, Research and Development expenditure), were identified as relevant to compare nano-enabled products with similar functionality or nano-enabled product with their conventional counterpart. The indicators are organized within a conceptual scheme comprising of benefits (Education, Employment and Professional training) and costs (Research and Development expenditure and Non-fatal accidents). A quantitative MCDA methodology is proposed and applied to a case study that compares the social impacts of two nanotechnology applications. One advantage of using MCDA with s-LCA is that qualitative stakeholder preferences on indicators can be combined with quantitative indicator scores. The gaps to be addressed to expand the future development of methodologies to assess social impacts of nano-enabled products are also considered in the work.

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Exploring correlation between spatial distribution of fertilizers emissions and social disfavour indicators: a case study in Luxembourg
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This work is the first step of a research aiming at a coupling of health geography and LCA to: 1) calculate and map the impacts of agricultural practices on ecosystems and 2) find their correlation with human factors that we call *social disfavour indicators* (such as low incomes, high distance from hospitals, low level of education, high rate of unemployment, etc.). The aim of the analysis is exploring the existence of a statistically relevant correlation between ecosystems' damages and human health, and providing a representation of this correlation at a fine spatial scale (at the level of the territory of single communes). In this respect, LCA can nicely complement health geography by providing spatial information (to calculate emissions and translate them into potential impacts) and health geography can provide to LCA powerful computational techniques to evaluate spatial correlations. In this presentation we show the application of a clustering methodology very commonly used in computational geography in order to groups communes by classes of criticality with respect to risk of environmental contamination and their correlation with variables of social disfavour. The presentation also introduces the limits of this method and proposes a possible enhancement of the methodology.

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Life cycle sustainability assessment for agricultural holdings: integrating environmental, economic and social aspects
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Despite the widespread acceptance of the 'three-pillar' model for sustainability – environmental, social and economic – there is still a serious lack of technically and practically feasible data-collection solutions for conducting a social life-cycle assessment (SLCA) and life-cycle costing (LCC) for a large sample of agricultural holdings. Despite extensive research there is still a considerable lack of methods for a comprehensive assessment of the sustainability at the farm level as many of them suffer from gaps in the conceptual framework and the thematic completeness. For these reasons, Agroscope has recently completed a project aiming at developing a scientifically sound set of indicators covering the most relevant aspects of sustainability in life-cycle thinking. This includes a comprehensive evaluation of the following impacts on sustainability: (i) resource use demand, (ii) global warming, (iii) eutrophication and acidification, (iv) ecotoxicity, (v) biodiversity, (vi) soil quality, (vii) economic sustainability, and (viii) social sustainability. A special focus was placed on the following aspects, which are highly relevant to agriculture: (i) ecotoxicity, biodiversity and soil quality, (ii) social sustainability, including animal welfare, physical and mental workload, and the aesthetic value of the agricultural landscape, and (iii) a set of economic indicators aiming to reflect the long-term economic viability of agricultural holdings. For the social dimension of sustainability, a detailed questionnaire was designed in order to address all dimensions of social responsibility, including interactions with business partners and the surrounding community, as well as with farm workers. Subjective social indicators were included in the indicator set with a view to supplementing the objectively derived indicators with perception-based information. Furthermore, a sophisticated

indicator for the workload of both the farming family and the employees was developed. The landscape quality will be assessed by the diversity Shannon index based on preference rating scores for typical agricultural landscape elements. Animal welfare will be included by a pragmatic approach based on a credit point system. The implementation of an efficient data collection process will be tested in a follow-up project based on a small sample of farms, with the mid-term objective to extend the sample on a substantial part of the Swiss farming sector.

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Life cycle sustainability analysis - a procedure and a case study
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This presentation aims to contribute to the development and demonstration of an operational approach to life cycle sustainability analysis (LCSA). This approach originates from the framework developed within the EU project CALCAS. The framework is different from the life cycle sustainability assessment outlined by Klöpffer in that it not only broadens the scope of life cycle assessment (LCA) to include economic and social aspects, but also allows for deepening of the analysis. It is also different in that it does not predefine the LCSA to be the sum of LCA, life cycle costing (LCC) and social LCA. Instead, the sustainability indicators, the systems investigated and the methods used for the analysis are all decided case by case. Our LCSA approach has two distinct features: 1. the case-specific research questions are defined in a participatory procedure that involves an Open Space workshop; 2. the analyses are carried through by a network of researchers and experts. A network is necessary because the research questions are not known in advance. We applied the approach in a sustainability assessment of a 50 km pipeline for transfer of residual heat from industries to a large district-heating system. The LCSA included 14 research questions on economic, environmental and social aspects. The results indicate that the pipeline is likely to reduce the total costs of the system, but the expected profit is rather small and uncertain, and it is difficult to find a market model that ensures eveyone a share of this profit. The environmental benefits of the pipeline are highly dependent on what electricity production increases when the use of residual heat in the DH systems reduces the combined heat and power production in these systems. The pipeline is likely to have no significant impact on the employment and a somewhat negative impact on the land owners. In conclusion, our LCSA approach proved to be operational. The Open Space format for workshops can generate a good basis for the research questions; however, care must be taken to ensure a balanced participation at the workshop, and complementary research questions might have to be added after the workshop. We found that an LCSA that is the sum of LCA, LCC and social LCA does not cover all sustainability aspects that stakeholders can consider important. We also found that the sustainability of a pipeline for residual heat is uncertain in this specific system and in the time frame investigated.

Exploring links between the biodegradation of chemical contaminants, the metabolic capability of microbial communities and environmental variables

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Biodegradation of volatile hydrocarbons in five surface waters tested as composed mixtures in the μg/L range
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Biodegradation is an important removal process for many chemicals that are released to the environment. High quality experimental data are thus needed for the development and verification of biodegradation models. In biodegradation tests as well as in the environment, the microbial population is not controlled, and therefore a source of variability. In this study the variability of biodegradation in five different surface waters in Denmark with different characteristics regarding pre-exposure to petroleum hydrocarbons was investigated. The study was conducted with a composed mixture of 9 hydrocarbons that were all within a narrow carbon number range (9-12 C) but diverse in molecular structure. The 9 tested compounds were characterized by low aqueous solubilities and even lower environmental concentrations, and all experiments were thus conduted well below solubility and in the μg/L range. The test method was based on OECD 309 but adapted for hydrophobic and volatile test chemicals. Stock solution of ~ 1/100 of the solubility for each chemical was prepared by partitioning based dosing from a loaded silicone polymer. 20 mL test systems were then prepared using 13.5 mL of the surface water innoculum and 1.5 mL stock solution. Test systems were incubated at 20 °C for a maximum of 28 days on a roller and at fixed time intervals three replicate test systems and abiotic controls were analyzed on GC-MS using fully automated Head Space Solid Phase Micro Extraction (HS-SPME). Primary biodegradation (substrate depletion) was determined based on the HS-SPME measurements relative to the abiotic controls. The general order of biodegradation in the five surface waters was n-Decane > Bicyclohexyl > 1,2,4-trimethylbenzene ~ Biphenyl ~ Naphthalene ~ Tetralin > 2,3-Dimethylheptane > Decalin > 1,3,5-Trimethylcyclohexane. Lag phases were between 0 and 8 days. The half-lives were similar in four of the surface water samples and lower than predicted using the BioHCwin model. However, 1,3,5-Trimethylcyclohexane had a predicted half-life of 3.5 days but was only degraded in one sample after all other test chemicals were degraded. In the fifth sample from the clean lake half of the half-lives were higher and half lower than predicted in BioHCwin.

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Exploring biotransformation of micropollutants in phytoplankton species: overview and influencing factors
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The role of phytoplankton in the biotransformation of micropollutants in surface water is largely unstudied; however recent studies indicate a potential role of phytoplankton organisms in the degradation of crop protection products. With three model species (*Microcystis aeruginosa* and *Synechococcus sp*., cyanobacteria, and *Chlamydomonas reinhardtii*, a green alga), we investigated biotransformation reactions of a set of 24 micropollutants, composed from strobilurin and azole fungicides representing diffuse source pollutants and pharmaceuticals representing point source pollutants. Among the reactions observed were hydrolysis of esters, dealkylations, methylations, deaminations and dehydrations, as well as some amino acid conjugations. Biotransformation activity was apparently not sensitive to the presence of low-concentration chemical stressors, however factors like pH and cell density effected some transformation rates. Selected reaction pathways, in particular associated with *Chlamydomonas* in single-strain experiments, could be observed also in experiments with phytoplankton communites sampled from a lake. The study provides a general overview over biotransformation reactions present in phytoplankton species and influence of experimental or environmental conditions.

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Glyphosate is biodegraded via two pathways in soil and water-sediment systems - a stable isotope co-labelling approach
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Glyphosate and its metabolite aminomethylphosphonic acid (AMPA) are frequently detected in soil and freshwater sedimentary environments, but there are no comprehensive studies on glyphosate behaviour in these systems. Microorganisms can use C and N from a pesticide to synthesize their biomass compounds, e.g. amino acids (AA) and fatty acids (FA). The extraction of known microbial biomarkers from soil or sediment after addition of C and N isotope tracer allows an estimation of microbial activity in the transformation of pesticide. We investigated the degradation pathways of glyphosate with the particular focus on the metabolic incorporation of the isotope label into AA, FA and their fate over time. An agricultural soil and water-sediment were incubated with co-labelled glyphosate ($^{13}C_3{}^{15}N$-glyphosate) in the dark and at constant temperature (20°C). $^{13}C_3{}^{15}N$-glyphosate was used to determine its turnover mass balance over a period of 80 days. Soil and sediment samples at the respective sampling date were analysed for the amount and the isotopic composition of AA, FA, CO_2, solvent-extractable parent compound and metabolites and total NER. In the water-sediment system, 55.7 % of ^{13}C of glyphosate was ultimately mineralised, whereas the mineralisation in the water system (without sediment) was low, reaching only 2.4 % of ^{13}C of glyphosate equivalents. Glyphosate was mineralised in the soil more rapidly and at the end labelled CO_2 constituted about 73% of $^{13}C_3$-glyphosate equivalents. A rapid increase in $^{13}C^{15}N$-AMPA after 10 days was noted in water-sediment system and these transformation products ultimately constituted 26.2 % of the $^{13}C_3$-glyphosate and 78.5 % of the ^{15}N-glyphosate equivalents. In contrast, in the soil, $^{13}C^{15}N$-AMPA increased initially but after 20 days decreased slowly reaching ultimately 12.3 % of the $^{13}C_3$-glyphosate and 39.6 % of the ^{15}N-glyphosate equivalents. Initially, glyphosate was biodegraded via the sarcosine pathway related to microbial growth, as shown by co-labelled $^{13}C^{15}N$-glycine. Later, degradation via AMPA dominated under starvation conditions, as shown by the contents of ^{13}C-glycine. The presented data provide the first evidence of the utilization of glyphosate as a C and N source and highlight the relevance of both the sarcosine and the AMPA pathways in the water-sediment and soil system.

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NfsA-FRP nitroreductase enzyme family: a new tool to link biotransformation pathways and environmental contamination by mesotrione, a beta-triketone herbicide
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Mesotrione is a β-triketone herbicide commonly used on corn since 2003. The fate and persistence of this compound in the environment is characterized by a relatively high mobility and bioavailability. Two major metabolites resulting from biotransformation are commonly found in soil: 4-methylsulfonyl-2-nitrobenzoic acid (MNBA) and 2-amino-4-methylsulfonyl benzoic acid (AMBA). Several mesotrione-transforming bacterial strains have been isolated from various environments such as farmland and cloud water. Although these strains belong to different genera, the mesotrione transformation pathways are well conserved and lead to MNBA and AMBA, as observed in soil. A mesotrione-transforming strain, *Bacillus megaterium* Mes11, isolated from farmland showing mesotrione transformation capacities [1] was used as a model strain to characterize its biotransformation pathways. Complementary chemical, biochemical and biological approaches were used to identify the biotransformation products of mesotrione and the enzymes involved in the first step of biotransformation. A proteomic study on Mes11 revealed a network of differential expressed proteins being linked to nitroreductase (NR) enzymes [2]. These results were consistent with the chemical structures of metabolites, strongly suggesting the involvement of a NR in the transformation of mesotrione into AMBA. A screening of Mes11 NR revealed two enzymes (NfrA1 and NfrA2) capable of reducing the nitro group of mesotrione. Both purified Mes11 NR were structurally and functionally characterized. They belong to the NfsA-FRP family of Nitro FMN reductases, previously shown as having activity on different nitro-aromatic compounds. Interestingly, members of the NfsA-FRP family have also been identified in other mesotrione-transforming strains, suggesting a key role of these enzymes for mesotrione transformation in various environments. The broad range of temperature and pH determined for Mes11 NR activities strengthened this hypothesis. This study constitutes the first identification of enzymes involved in mesotrione biotransformation. These enzymes (or the corresponding genes) may be used as biomarkers to predict natural attenuation of mesotrione in the environment and to assess potential contamination by the parent molecule and/or metabolites. NfsA-FRP NR could also be used in bioremediation processes or in the development of *in situ* biosensors for detection of nitro-aromatic compounds.

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Effects of Solids Retention Time on Micropollutant Biotransformation Rates and Pathways in Activated Sludge
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An important parameter in wastewater treatment is the solids retention time (SRT), defined as the time during which the suspended biomass, on average, is retained in the system. Since slow-growing prokaryotes such as nitrifying bacteria can only build up significant populations at higher sludge ages, the SRT significantly influences the microbial community composition. Several research groups have reported an enhanced micropollutant removal capacity of different compounds at higher SRTs, e.g., [1], the underlying causes however are still unclear. In this study, a series of six sequencing batch reactors (SBRs) was operated at SRTs between one and fifteen days. With batch experiments, the biotransformation rates (k_{bio}) were estimated from the concentration-time series, and transformation product formation was investigated for compounds belonging to specific chemical classes of interest. Removal of ammonia and the formation of nitrate were higher in the SBRs run at higher SRT. The estimated biotransformation rate constants from the batch experiment were normalized for each compound and when these normalized rate constants were averaged across all compounds for each reactor, a strong correlation between the average rate constant in each reactor and SRT was observed. However, we found that individual compounds showed strongly deviating behavior from this average trend. A clustering of compounds according to the trends of the transformation rate constants with SRT was therefore sought. When doing so, we observed different trends amongst the compounds, three of which correlated well with nitrification rate, oxygen uptake rate and SRT, respectively. For three of the clusters we identified groups of chemically similar compounds that (mostly) showed the same trend. The group of p-aminobenzenesulfonamides, for instance, showed a slight decrease of transformation rate constants with SRT, highlighting the importance of understanding the influence of SRT at the level of compound classes. In a follow-up analysis, we will then attempt to link the observed trends for types of biotransformation reactions with microbial community features, including taxonomic richness, composition and gene expression of relevant functional genes. References: [1] Clara M., *et al*. 2005. Water Research 39(1), 97-106.

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Preliminary biodegradation studies in water of commercial homo-polymer polycaprolactone by MALDI-TOF IMAGING technique to be used as probe of river metabolism
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MALDI IMAGING MS consists on the use of matrix-assisted laser desorption ionization as a mass spectrometry imaging technique. The sample is scanned in two dimensions at a preselected spatial resolution while the mass spectrum is recorded. Advantages, like measuring the distribution of a large amount of analytes at one time without destroying the sample, make it a useful method in solid-surface study. The present study aims at developing an analytical quantitative method for the study of biodegradation of a commercial homo-polymer to be further used as a probe for the evaluation of the so called "river metabolism". Polycaprolactone (average MW=1250) was selected as suitable candidate polymer. MALDI-ToF imaging experimental conditions were optimized. The method was tested in the lab using under different environmental conditions. Progress of biodegradation after several days of exposure was reflected on the changes in the mass spectra corresponding to variations in the chain length distribution pattern. Results were investigated using different image processing tools

Mechanistic toxicology of engineered nanomaterials: state of the art and future perspectives (II)

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Toxicity exerted by AgNPs in OECD and LUFA 2.3 soils: From molecular to organism level responses in Eisenia fetida earthworms
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Over 400 consumer products contain AgNPs (24% of the total products containing nanomaterials; e.g., detergents, paints, printer inks and textiles), which are also used in biomedical devices and water treatments due to their antimicrobial properties. Since the number of applications is widely increasing the release of AgNPs into different environmental compartments is already occurring, thus, the concern about the still scarcely known hazards of nanosized materials to environmental health has considerably grown. To date, the potential risk of AgNPs has been mainly studied in aquatic environments, while their effects on soils are less investigated despite the great complexity of soil matrix and the potential interactions of soil components with pollutants. These components (O.M, cations, soil colloids and water) together with the soil type varying pH and ionic strength factors, may affect the behavior of NPs, with particular effect on the aggregation and subsequent effect on their toxicity in organisms inhabiting soils. *Eisenia fetida* earthworms have been widely used as model organisms for soil health assessment due to their capacity to accumulate pollutants and their quick and measurable responses at different levels of biological complexity. Hence, the aim of this work was to compare the toxicity exerted by AgNPs in two widely used standard soils through molecular, biochemical and cellular responses measured in this earthworm. With such a purpose, *E. fetida* earthworms were maintained in OECD artificial soil (10% O.M) and in LUFA 2.3 natural standard soil (1.62% O.M) spiked with environmentally relevant and high but sublethal concentrations of AgNPs (0, 0.05 and 50 mg AgNPs/Kg). After 3 and 14 d of exposure, Ag accumulation, weight loss, coelomocyte number and viability, metallothionein (MT) levels and catalase (CAT) activity, damage in DNA and MT transcription levels were recorded. Results indicated that earthworms exposed to 50 mg AgNPs/Kg presented the highest weight loss, affection on coelomocyte parameters, an increase in MT levels and CAT activity and DNA damage in both soil types, although these effects were enhanced in earthworms maintained in LUFA 2.3, where mortality (7,5-10%) was also observed. It can be concluded that apart from the intrinsic properties of NPs (shape, size, surface chemical and charge) the characteristics of the exposure scenario (i.e. soil type, pH, O.M) are to be considered when assessing toxicity of AgNPs in soil.

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Toxicity of Pristine and Aged Coated Copper Oxide Engineered Nanomaterials (CuO ENMs) to the Earthworm E. fetida
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Many engineered nanomaterials (ENMs) are coated to enhance their technical properties, but these coatings may also change their environmental fate and potential toxicity. There is currently little knowledge on the biological effects of aged nanomaterials and therefore two experiments were conducted to assess the differences in the effects of different coatings on ENMs to adult earthworms (*E. fetida*) as pristine ENMs and after 1 year of aging based on OECD TG 207. In both studies, worms were exposed for 14 d to negatively/positively charged, organic coated or uncoated (core) CuO ENMs (indicative size range of primary particles 10-20 nm) at nominal concentrations of 200 and 1000 mg Cu kg^{-1} with a comparison to ionic copper (200 mg Cu kg^{-1},as $CuSO_4$) in standard Lufa 2.2 soil. Survival, weight of earthworms and tissue metal concentrations (after a 24 h depuration period) were assessed at 7 and 14 d in each experiment. Biochemical endpoints were also analysed in worms from both experiments at 14 d to enable the determination of oxidative and osmoregulatory stress. Total Cu concentrations and pH (in 2:1 soil:water mixture) were measured in soils at 0, 14 d and after 1

year of aging. There were no statistically significant differences between soil pH in all treatments (ANOVA, $P > 0.05$) so the data were pooled and the pH was 5.3 ± 0.2 and 5.2 ± 0.3 (mean ± SD, $n = 40$) at the end of the pristine and aged soil experiment, respectively. After 14 d, control group survival was 100 % in both pristine and aged soil experiments. In worms exposed to 1000 mg Cu kg^{-1} CuO ENMs, survival was only 50 and 40 % in the negatively and positively charged CuO ENM treatments, respectively, while in aged soil experiment only exposure to positively charged CuO ENMs resulted in a 30 % survival. In both pristine and aged soil experiments, body wet weights were significantly reduced (ANOVA, $P < 0.05$) between 10 and 30 % and 30 and 50 % in all low and high Cu treatments, respectively, compared to controls. Total Cu in earthworms exposed to 200 mg Cu kg^{-1} and 1000 mg Cu kg^{-1} ranged from 0.02 – 0.14 and 0.05 to 0.3 mg Cu kg^{-1} dw, respectively, at the end of the pristine soil experiment, analysis of the worms from the aged soil experiment is in progress. Early findings indicate aged ENMs are less toxic to earthworms than the pristine ENMs. The research is funded by EU FP-7 NANOSOLUTIONS Project, Grant Agreement No.309329.

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Toxicity of silver nanoparticles to soil organisms: an integrated in vitro- in vivo approach

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As engineered nanoparticles (ENPs) continue to find increasing applications in society, elucidation of their potential to pose environmental risks is urgent. We investigated the effects of size (20, 35 and 50 nm) as well as surface coating/charges (chitosan/positive CHIT; bovine serum albumin/negative BSA, and polyvinylpyrrolidone-55/neutral PVP-55) on the toxicity of silver nanoparticles (AgNPs) in *in vivo* (*Lumbricus rubellus*) and *in vitro* (RAW 264.7 cell line) models. A 28-day sub-chronic exposure in soil spiked with varying concentrations (0 – 250 mg/kg soil) revealed highest uptake of AgNP_BSA by earthworms (body burdens 50 – 100 mg/kg BW), with already high uptake after only 72h. The number of cocoons produced was most affected by negatively charged AgNP_BSA particles. Similarly, results from cellular *in vitro* tests indicate the highest induction of TNFα with AgNP_BSA (70x higher than control). Significant *in vitro* ROS induction was only observed for the 20nm positively chitosan coated particles. Cytotoxicity tests showed no significant differences between the different types of AgNPs. Gene expression profiling using whole tissues of earthworms exposed to the different AgNPs was conducted and analyses are on-going. Early indications of gene alterations suggest the AgNP_BSA to be more effective. The present study provides further evidence of the influence of ENP properties in driving their bioaccumulation and toxicity.

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Trophic transfer of engineered nanoparticles in terrestrial food chains

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The trophic transfer of engineered nanoparticles from soil through terrestrial food chains is being evaluated. Cerium oxide and lanthanum oxide bulk and nanoparticles (NP) were added to soil (0-1000 mg/kg) with zucchini or lettuce plants, respectively. Additionally, studies have been initiated in which lettuce was added to soil 0 or 70 d after amendment with copper oxide NP, bulk and ions (0-400 mg/kg). The plant Ce, La and Cu accumulation was determined by ICP-MS and leaves were fed to crickets or darkling beetles for 14 days. Crickets were then fed to secondary consumers; wolf spiders, mantids or lizards for 7 or 14 days. The Ce, La and Cu accumulation in the primary/secondary consumers and cricket/lizard feces were also measured. Results from the Ce experiment showed that zucchini Ce content was significantly greater with the NP exposure. The flowers, leaves, stems, and roots of bulk exposed plants contained 93.3, 707, 331, and 119,000 ng/g, respectively; nanoceria-exposed plants contained 153, 1510, 479 and 567,000 ng/g, respectively. Crickets fed bulk and NP-exposed leaves contained 15 and 34 ng/g, respectively. Spiders consuming bulk-exposed crickets had non-detectable Ce but NP-fed spiders contained Ce at 4.9 ng/g. Upon lanthanum exposure, lettuce mass was reduced across all treatments but there was no difference in lettuce La content based on particle size. Although the CuO experiment is ongoing, preliminary results from pigment content/production showed no significant effects in lettuce from the two soils (0 or 70-d weathered Cu) upon Cu treatments exposure. Interestingly, expression levels of several target genes involved in Cu transport suggest that the mechanisms involved in CuO NPs accumulation are differentially regulated as compared to ionic Cu. Additional results focused on accumulation of Cu in plant, crickets and lizard tissues will also be presented.

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Approaches towards predicitng the tecotoxicity of nanomaterials

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Nowadays, nanomaterials are highly integrated into our daily life. However, recent studies have shown obvious toxicity of some nanoparticles to living organisms, and their potentially negative influence on environmental ecosystems. The goal of the present study was to develop predictive models that allow to efficiently predict the ecotoxicological properties and effects of inorganic nanomaterials (metals and oxides). The numerical data on toxicity of nanoparticles to different organisms have been taken from the literature and were uploaded in a data base. The main characteristics of nanoparticles such as chemical composition of nanoparticles, average particle size (APS), shape and information about the biological test species were used as obligatory condition for all properties in the data base. 20 QSAR-type of models were compared by following the same procedure with different combinations of descriptors and machine learning methods. The QSAR methodologies applied, used Random Forests (WEKA-RF), k-Nearest Neighbors and Associative Neural Networks. The predictive ability of the models was tested through leave-one-out cross-validation, giving a $q^2=0.69$-0.78 *for regression models and total accuracies Ac=73-99% for classification models. Predictions for external evaluation sets obtained accuracies in the range of 69-85% (for low/high toxicity classifications) and* $q^2=0.70$-0.78 *for regressions.The method showed itself to be a potential tool for estimation of toxicity of new nanoparticles at early stages of nanomaterial development.*

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QSAR Models for heterogeneous Nanoparticles: Results from the COST Action TD1204 MODENA - WG3 Exercise

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This study shows partial results generated by the author within the context of the COST Action TD1204 'Modelling Nanomaterial Toxicity' (MODENA). This action was launched in 2012, and generated a network of scientists currently active to promote trans-disciplinary cooperation on the topic of computational nanotoxicology. A main output of this cooperation has been the creation of a large dataset with cytotoxicty data measured in different cell lines for heterogeneous nanoparticles (NPs) by 9 laboratories operating within MODENA. This dataset was shared among members of the Working Group 3 (WG3 - modelling) in order to investigate the possibility to generate models on such dataset. This dataset included 192 heterogeneous NPs representative for 11 different metals and materials (i.e. Ag, Au, Fe, Co, Ni, Si, Ce, C, Ti, Zn, PLGA-PEO), structurally characterized by experimentally measured properties, such as shape, size, surface area etc.. Approaches based on Quantitative Structure Activity Relationships were applied to predict different responses of biological activity represented by EC_{25} and EC_{50} values measured in human cell cultures, using four cytotoxicity assays (i.e. ATP assay, LDH assay, MTT assay, and WST-1 assay). The models were developed by using the software QSARINS by Multiple Linear Regression (MLR - Ordinary Least Squares (OLS) method), performing exhaustive search i.e. by exploring all the possible combinations of variables. The best models were chosen as those with the best statistical performances according to various validation parameters. This study confirmed the scientific relevance of the dataset created within MODENA starting from data generated independently by different laboratories, and without a priori supervision. The coherency of the information included in the data set was captured by the modelling approach which identified robust structure-activity relationships on the basis of interpretable descriptors. A main issue emerged regarding the dependency of the quality of the models on the metric used to express toxicity. Results show that the metric "number of NPs/ml" gives the best modelling performances, and thus may be suggested as preferable metric to generate QSAR models for heterogeneous datasets.

Polar ecotoxicology: hot issues in cold climates!

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Influence of environmental and ecological factors on plasma perfluoroalkyl substances (PFASs) in female polar bears (Ursus maritimus) from the Barents Sea

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Perfluoroalkylated substances (PFASs) are synthetic chemicals used for various industrial purposes since the 1950s. The most commonly detected groups of PFASs in biota are perfluoroalkylated sulfonic acids (PFSAs) and perfluorocarboxylic acids (PFCAs). Many PFASs are highly persistent and have similar biomagnification capacity as lipophilic persistent organic pollutants (POPs), such as polychlorinated biphenyls (PCBs) and chlorinated pesticides in Arctic marine food webs. In contrast to PCBs and chlorinated pesticides, PFASs have strong affinity to protein-rich tissues such as liver and blood. The concentrations of these emerging compounds in Arctic marine predators such as polar bears (*Ursus maritimus*) are comparable or higher than PCBs and chlorinated pesticides. The high concentrations of PFASs in Arctic apex predators are of great concern due to their potential health effects such as metabolic disruption, developmental toxicity, thyroid disruption, immunological effects and neurotoxicity. Throughout their life cycle, polar bears alternatively accumulate and lose massive fat depots following the fluctuations in accessibility of their prey. In this context, the current study aimed at examining the environmental (i.e., season, year and diet) and ecological factors (i.e., breeding status and space use strategy) on plasma PFASs in female polar bears from the Barents Sea. To do so, we sampled females with variable reproductive status (i.e., solitary, with cubs of the year or with yearlings) over two seasons (spring and autumn) and two years (2012 and 2013). The concentrations of only three compounds were influenced by the season (PFNA, PFDA, PFTriA) and one compound by the breeding status of females (PFTriA). All PFASs compounds were explained by a dietary tracer ($\delta^{15}N$ RBC) implying an influence of diet on PFASs exposure. Moreover, $\delta^{15}N$ RBC and most compounds were significantly influenced by the space use strategy of females with pelagic females showing higher $\delta^{15}N$ RBC and higher plasma concentrations of PFASs compared to local females. Our results suggest that the influence of dietary exposure on plasma concentrations of PFASs is driven by the space use strategy of females. This further suggests a possible indirect role of climate change in PFASs exposure through alterations in migration patterns of polar bears (i.e., the ecology of species).

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How do pollutants and fat get along? Insights in a "feast and fast" specialist, the polar bear.
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Changing climate will challenge arctic animals energetically. For example, prolongation of ice-free summer seasons will lead to prolonged fasting periods of polar bears, which use sea ice as a platform for hunting. Fasting is physiologically demanding and requires optimal control of energy homeostasis. Energy deficiency places greatest demands for reproducing females and may ultimately affect reproductive success and population recruitment. Peroxisome proliferator-activated receptors (PPARs) play a central role in regulating energy homeostasis. Recent research indicates that several currently used chemicals, may disturb the function of PPARs. We hypothesize that contaminant exposure may affect the energy homeostasis of polar bears and thus decrease their ability to respond to climate change. To explore our hypothesis, we measured PPARγ mRNA expression in adipose tissue and related it to legacy chlorinated and brominated pollutants. PPARγ mRNA expression was positively related to most pollutants. We also analyzed metabolomics, which is a comprehensive study of low molecular weight water-soluble metabolites. This methodology can give mechanistic insight into changes in metabolism due to a stressor such as fasting. We tested the relationships between metabolome and fasting, as depicted by the urea/creatinine ratio in plasma. We observed a negative relationship between metabolome (characterized by lipid fingerprints) PCA scores and urea/creatinine. Additionally, we observed a positive relationship between lipid fingerprints PCA scores and ΣChlordanes in autumn but not spring. Consequently, our results suggest that several pollutants can disrupt lipid storage and possibly lipid stores' functionality, this could impair the ability of polar bears to adapt to climate changes.

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Lipophilic Persistent Organic Pollutants as Indicators of Adiposity in Humpback Whales
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Southern hemisphere humpback whales are naturally obese mammals, highly adapted to the productivity extremes of Polar latitudes. Multiple research disciplines are alerting us to progressive weight loss and changing foraging habits in these populations, signalling underlying ecological change. Despite the potential gravity of this trend, unravelling the nutritional signals of humpback whales to provide an unambiguous evaluation of species energy reserves, and therefore vulnerability to ecological change, is hindered by the inadequate power of existing non-lethal, cetacean research approaches. Humpback whale external blubber adipocyte area analysis has recently found, by the investigative team, to be adequately powerful to distinguish between “fed” and “fasted” migrating cohorts. Similarly, the investigative team has previously shown the dramatic concentration effect that occurs in outer blubber Persistent Organic Pollutant (POP) levels between the two migrating cohorts, lending support for both paramters as indirect measures of energetic reserves. Here we demonstrate inter-annual fluctuations in energetic reserves as revealed by these parameters in combination with stable isotope analysis. We advocate the scrutiny of POP burdens in long term monitoring programs, as indirect indicators of energetic health.

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From Antarctica to the subtropics: latitudinal differences in trace element and organic pollutant contamination in Southern Ocean skuas (Catharacta spp.)
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Polar seabirds integrate bioaccumulative contaminants via food intake and have revealed geographical and temporal trends of contamination, especially in the Arctic. By contrast, spatio-temporal patterns of contamination of Antarctic and subantarctic food webs are still poorly known. Pre-fledging seabird chicks are particularly interesting as bioindicators of food web chemical contamination, because their food and their parents’ foraging ecology during the chick-rearing period are relatively well-known. Hence, concentrations in chick tissues are more easily related to dietary sources and represent primarily the local environment. Here we measured mercury (Hg) and 13 other trace elements, and persistent organic pollutants (POPs, including organochlorine pesticides and polychlorinated biphenyls) in blood of skua chicks from four breeding locations that encompass a large latitudinal range in the southern Indian Ocean: from Antarctica (Terre Adélie, Catharacta maccormicki), through subantarctic areas (Crozet and Kerguelen Islands, C. lonnbergi), to the subtropics (Amsterdam Island, C. lonnbergi). Concentrations of the highly-toxic Hg increased from chicks in Antarctica to chicks in the subantarctic and subtropical islands, with a factor of eight between the populations with the lowest and highest burdens (0.5 ± 0.1 vs. 4.0 ± 0.8 $\mu g\ g^{-1}$ dry weight, mean ± SD, at Terre Adélie and Amsterdam Island, respectively). Selenium (Se) concentrations were extremely high in some skua populations, reaching levels never shown before in any bird species. Se was positively associated to Hg, suggesting its involvement in protective mechanisms against Hg toxicity. Chicks’ POP pattern was largely dominated by pesticides, in particular DDT derivatives and hexachlorobenzene (HCB). Overall, POP concentrations were low, although HCB levels were comparable to related species from the Northern Hemisphere. Skua chicks from the subtropical site had lower levels and diversity of POPs than those of the subantarctic and Antarctic populations. The strong inter-site differences in Hg and POP levels highlighted in the present study are consistent with previous results showing a latitudinal trend in the incorporation of these contaminants in Southern Ocean food webs. The skua populations studied here could serve as useful models to investigate the potential effects of these contaminants on seabird physiology and demography.

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Contaminant loads in relation to wintering area and breeding habitat in the Arctic Skua
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Seabirds are often used as model species in ecotoxicological studies as they are near the apex of most marine food chains, and therefore bioaccumulate high concentrations of certain pollutants. Most seabirds that breed in temperate and Arctic regions are migratory, and therefore spend a considerable part of the year away from their breeding areas thereby functioning as biovectors. Several studies have implied that migration may have considerable effects on seabird ecotoxicology. In this study, we have studied migration strategies in the arctic skua *Stercorarius parasiticus*. It breeds in arctic and subarctic areas, and in Svalbard and Northern Norway, it spends the nonbreeding part of the year (September through May) away from the breeding grounds. We have used Global Location Sensor (GLS) loggers to track the migration strategies of the same individuals over several years. This has revealed different individual wintering areas spanning large parts of the Atlantic Ocean. We report that the individual birds repeatedly target the same winter area year after year. We have analyzed various pollutants such as heavy metals and PCBs to look for individual differences in contaminant load related to different wintering areas and breeding habitat in the Arctic.

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Do reproductive strategies affect pollutant levels in pinnipeds? A meta-analysis
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In highly seasonal environments where food availability can be limited, such as the Arctic, animals depend on the use of stored energy reserves. Species store energy as lipids which they can allocate towards survival, growth and reproduction. Animals that store energy prior to reproduction are described as â_capital breeders’, and those that feed during reproduction as ‘income breeders’. In marine mammals such as seals, breeding substrates (fast ice and land vs. drift ice) and access to food resources during the breeding period can determine which

strategy a species may adopt. In polar habitats such as the marginal ice zone where ice is unstable and food is temporally limited, capital breeding is favoured. However, the use of lipids means that persistent organic pollutants (POPs) are also stored, remobilised and maternally transferred during reproduction, especially during lactation. Most studies on maternal pollutant transfer have investigated only single species, meaning that a study synthesising the overall effect of life history strategy on the bioaccumulation pollutants is lacking. A meta-analysis was conducted to test the effects of reproductive strategy (income and capital breeding) on pollutant levels in mother-pup seal pairs. Reproductive strategy was measured as pup daily mass gain during lactation, with capital breeding pups gaining more weight per day than income breeding pups. The meta-analysis showed that capital breeding mothers transferred more lipid soluble POPs such as polychlorinated biphenyls (PCBs) to their pups than income breeders. On the other hand, protein associated pollutants such as mercury (Hg) was not transferred as efficiently by either reproductive strategy, compared to lipid soluble POPs. Studies with data on unrelated juveniles and adult females in the same population were also compared and pup daily mass gain was found to be a poor descriptor of pollutant levels across seals, most likely because using direct mother-pup seal pairs are best suited to studying the effects of reproductive strategies. In summary, offspring of capital breeders are at greater risk to negative health effects as they are exposed to higher levels of pollutants than income breeders, given similar POP levels across female seal species. These findings are of particular importance in polar regions, where species have adapted to utilise stored energy reserves, and are especially relevant when considering how these areas are affected by climate change.

Soil and water contaminants: evaluation, biomonitoring and bioindicators for effective management (II)

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Nutrients in urban surface waters: urban aquatic ecosystem threat and water re-use

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Introduction Domestic and industrial discharges into urban surface waters including streams and rivers can result in nutrient enrichment, de-oxygenation and reduction of freshwater quality. This could lead to increased hazard in waste water re-use, and threat to urban aquatic ecosystem diversity and human health. This study investigates the levels of dissolved oxygen (DO), oxygen demand (BOD, COD), inorganic nutrients ($N\text{-}NO_3^-$, $N\text{-}NO_2^-$, $N\text{-}NH_4^+$ and $P\text{-}PO_4^{3-}$) and organic carbon (OC) in Ogunpa River (an urban surface waters) in Ibadan metropolis, Oyo State, Nigeria. **Materials and methods** Water samples were pooled at the Ogunpa River source and at different sampling stations in different area location along the Ogunpa River. The water samples were prepared and analysed using standard methods. **Results and discussion** pH Organic carbon % DO mg/L BOD mg/L COD mg/L $N\text{-}NO_3^-$ mg/L $N\text{-}NO_2^-$ mg/L $N\text{-}NH_4^+$ mg/L $P\text{-}PO_4^{3-}$ mg/L Minimum 6.8 2.1 2.85 7.39 2.11 7.34 6.19 0.085 2.83 Maximum 7.4 3.6% 5.37 10.52 8.67 9.58 7.85 0.51 9.68 The DO, BOD, COD ranged, 2.85 – 5.37 mg/L, 7.39 – 10.52 mg/L, 2.11 - 8.67 mg/L respectively. Organic carbon and pH ranged 2.1 – 3.6% and 6.8 – 7.4 respectively, while $N\text{-}NO_3^-$, $N\text{-}NO_2^-$, $N\text{-}NH_4^+$ and $P\text{-}PO_4^{3-}$ were, 7.34 – 9.58 mg/L, 6.19 - 7.85 mg/L, 0.085 – 0.51 mg/L, and 2.83 - 9.68 mg/L respectively. The high nutrient concentrations of the water, may be attributed to unsustainable release of high nutrient streams and disposed solid wastes. This results in the undesirable effects of corrosion and incrustation observed in most sampling stations, leading to the drop in DO and thus the high COD and BOD. **Conclusion** In effect, the self-purification and regeneration capacity of the water is low due to consistent exposure. This suggests high cost of water treatment for re-use. An appropriate monitoring and management procedure is therefore required to improve the water quality, and sustain the ecosystem.

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New tool of multi component three-phase exposure: Bioavailability and mobility of organic contaminants in solid phase samples

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The bioavailability plays crucial role in ecological risk assessment of soil contaminants. There are two approaches of environmental risk assessment, the biological and chemical, which complement each other. We developed a novel approach for rapid assessment of bioavailability and potential mobility of soil contaminants. The response of the same test organism to the organic extract, water extract and solid phase of soil was recorded and compared. This approach was designed to give an initial estimate of the total organic toxicity (response to organic extractable fraction), as well as the mobile (response to water extract) and bioavailable fraction (response to solid phase) of soil samples. The selected soil samples with different level of contamination (PAHs, PCBs, HCH and DDT) and different amount of organic carbon was used to demonstrate the efficiency of proposed method for analysis of contaminants fractionation in solid samples. The results showed a low potential mobility of organic toxicants in tested soils. The bioavailable fraction was significantly greater than the mobile fraction. Still, the large fraction of overall contamination was strongly bound to the solid samples showing significant dependence on the amount of organic carbon. The fraction bioavailable to the test microorganism was likely elevated by the effect of driven desorption. The presented new tool of "multi component three-phase exposure" utilise the exposure of the same test organism and it is useful for evaluation of total toxicity, water soluble and biologically available fraction of the solid sample contamination. The three-step ecotoxicological evaluation provides early information about behaviour of contaminants in soils in the context of mobility and bioavailability. The knowledge of mobility and bioavailability of chemical compound in a soil is useful for the hazard identification (provides useful toxicity and exposure information) and environmental risk assessment for organic contaminants in soils.

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Novel β-FeOOH/NiO composite material as potential catalyst for ozonation of organics

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This study reports the on novel synthesis of an organic linker mediated nano composite comprising of β-FeOOH and NiO and its possible application to heterogeneous ozone catalysis. The synthesized composite material was characterized using XRD, FTIR, TEM. Elemental mapping of single composite material under dark field scanning mode confirmed the presence of both Ni and Fe in the composite. 4-chlorophenol (4-CP) was used as target molecule to evaluate the catalytic activity. Above 5 % β-FeOOH loading on NiO, catalytic efficiency reduced. The composite material was stable under acidic (pH 2.5) and alkaline (pH 10), with the resulting solutions having no traces of Ni or Fe as evident from AAS measurements. Catalytic ozonation removed 85 % of 4CP in 20 minutes compared to 47 % removed by ozonation alone. The catalyst showed good recyclability as the catalytic activity of the material could be restored after calcination. The catalytic activity of the composite was due to the higher generation of $OH^?$ as shown by photoluminescence experiments. This novel composite material therefore shows as a future catalyst in water purification processes.

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The sorption of active pharmaceutical ingredients onto soils under different pH and microbial conditions

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Population growth, increasing affluence, and greater access to medicines have led to an increase in active pharmaceutical ingredients (APIs) entering sewage networks. In the low and lower-middle income countries (LLMIC) of Asia, Africa and Central and South America, the use of human pharmaceuticals increased by 23-29 % between 2000 and 2011. As a consequence, the loadings of residual APIs and other down the drain chemicals (including personal care products (PCPs) and cleaning agents to soils, surface and ground waters of these countries are increasing. In areas with high wastewater reuse residual quantities of APIs may enter agricultural soils via irrigation with treated, partially treated, or even untreated wastewater and sludge. Wastewater used for irrigation is currently not included in chemical environmental risk assessments (ERAs) and requires further consideration in areas with high water reuse. The aims of this research were to measure the influence pH and the soil microbial community has on the sorption of three APIs to soil. Propranolol, naproxen and ofloxacin were chosen as the APIs to focus on in these experiments as they cover a wide range of API characteristics and are widely used throughout LLMICs. The OECD 106 technical guideline was used to generate data on the adsorption and desorption kinetics for the three APIs using two soil types under natural, pH altered and sterilised soils. Future work as part of this project will include applying minimally treated wastewater to the soils and measuring how API sorption and degradation is affected and column experiments to assess leaching potential to groundwater sources. The data gathered through these experiments will aid the development of robust ERAs for APIs in soil for wastewater irrigation.

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Fate of metamitron in soil - a detailed insight into its biotransformation pathways and biogenic residues formation

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Metamitron (MTR) is commonly used in sugar beet crops and has received great concern due to its wide application. Although many studies addressing MTR behaviour in the soil systems are available, the detailed insight into its microbial turnover is still missing. We investigated biotransformation of MTR with the particular focus on the metabolic incorporation of the isotope label into FA and AA and their fate in soil over time. In biotic system, the mineralization of MTR started immediately without any lag reaching finally 60% of the initial $^{13}C_6$-metamitron equivalents. In contrast to biotic system, abiotic system showed a lower mineralization, amounting 7.4% of the initially added $^{13}C_6$-metamitron MTR was degraded initially to desamino-metamitron in soil and water-sediment might be assigned to microbial growth. Desamino-metamitron is ultimately converted to pyruvate and acetaldehyde. Pyruvate is used for direct synthesis of alanine as proven by the dominant presence of ^{13}C-alanine throughout the experiment, including both growth and starvation conditions. In addition, pyruvate and acetaldehyde entering the tricaboxylic acid cycle contributed indirectly to glutamate synthesis via 2-oxoglutarate and may explain high contents of ^{13}C-labelled glutamate. The complete dissipation of desamino-metamitron from soil after 16 days together with a rapid mineralisation of MTR leaving only traces of MTR in soil on day 16 suggests the relevance of this pathway in active metabolism. Based on the measured content of total AA (14.85% of $^{13}C_6$-metamitron equivalents) we could estimate that 29.7% of carbon-derived MTR was assimilated by microorganisms. At the end, ^{13}C-label derived from MTR was distributed mainly between $^{13}CO_2$ and microbial biomass indicating a complete degradation of this herbicide in soil. Using stable isotope label tracer (^{13}C), we established a detailed MTR turnover mass balance and metabolic fate analysis including biogenic residues formation. The presented data showed that nearly all NER were biogenic and were based on non-toxic biomass compounds stabilized in the SOM. In addition, we proved for the first time that MTR was utilized as a C source and was biodegraded via two pathways the "desamino-metamitron" and via the "Rhodococcus pathway".

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Threatened southern African soils: Status and challenges from an (eco)toxicological risk assessment perspective
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Soils in Southern Africa (SA); comprising South Africa, Namibia, Botswana, Zambia and Zimbabwe are, in terms of area, not a limited resource but are in terms of arability. This is due to limited rainfall in the area and inherent poor physical condition of these soils. As in the rest of the world soils in SA are threatened by a range of anthropological activities e.g. mining, agriculture and industry. Ecotoxicological risk assessment (RA) of soils has been well studied worldwide. Although some risk assessment of soils has been done in SA it has not been extensive. Soil screening values exist in South Africa, which enables soil quality assessment, but lack a clear risk-based scientific foundation and site-specific risk assessment. This is especially important because of the vast mining activities and the proximity of mine tailings disposal facilities to residential areas; exposing people living close by to a wide range of possible contaminants. We ask the question; to what extent the developed general RA systems used in other parts of the world are valid for the specific conditions in SA? We also investigated the status of the (eco)toxicological research in SA. This presentation intends to give an analysis and overview of the present situation, highlight the impacts on soil in SA, and assess current research as well as identifying knowledge needs and gaps.

Multiple stresses in aquatic ecosystems: Assessment of stress response and its consequences in organisms (II)

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Cross effects of salinity and ceria nanoparticles on two endobenthic bivalves Corbicula fluminea and Scrobicularia plana: multibiomarker assessment
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Cerium nanomaterials (CeNMs) are integrated in numerous consumer products (e.g. wood stain, painting, fuel additive) because of their strong abrasive power, high resistance and UV-shielding properties. Given the production volumes of CeNMs and their potential release into the aquatic environment, assessment of the potential environmental risks of CeNMs is a priority. While the impacts of Engineered Nanomaterials (ENMs) in freshwater media have been largely studied this last decade, data covering a salinity gradient are scarce. The aim of the present study is to assess the fate, behaviour and toxicity effects of both CeNMs included in a commercial fuel additive (Envirox™) at two stages of its life cycle and of NM-212, a standardized CeNMs provided from Joint Research Centre, across a salinity gradient (1.5, 15.0 and 30.0 practical salinity unit) during 28 days. Two euryhaline bivalves were selected: Corbicula fluminea and Scrobicularia plana, representative of freshwater and estuarine environments, respectively. Both species are well recognized as good models for biomonitoring purposes. These filter-feeding species may be particularly at risk of ENM exposure since they live at the water-sediment interface. C. fluminea were exposed to a constant water volume at 1.5 and 15 psu; while S. plana were exposed to an artificial rhythm of tide at the laboratory (6 h high tide/ 6 h low tide) at 15 and 30 psu. The contaminants were added gradually every 3 days (90 μg Ce /L) during the whole duration of experiment. The size, shape and cristallography of the uncombusted and combusted Envirox™ were performed by Transmission Electron Microscopy (TEM). Total Ce in the water column, labile forms of Ce accumulated in Diffusive Gradient Thin film (DGT) tools and Ce concentrations in the digestive glands of bivalves were estimated by ICP-MS. After 7, 14, 21 and 28 days of exposure, individuals were submitted to burrowing tests. Toxicity responses of both bivalve species were evaluated using a multi-marker approach at different levels (sub-cellular, individual) of biological organization using an adjustable pocked-size marine and freshwater mesocosm platform. Results showed in both species a significant difference of biomarker responses according to salinity, exposure condition and exposure time. Moreover, the differences between the conditions of exposure were discriminated with a reduced set of biomarkers: cellular damage, antioxidant defences, cellular respiration condition.

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Environmental health assessment of the Seine estuary using a battery of indicators at different levels of biological organization
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It is estimated that the global economic scale of environmental damage due to anthropogenic activities in coastal zones and estuaries is approximately $12.6 trillion per year. The Seine estuary is of particular concern as it is influenced by twenty-five percent of France's population and forty percent of the countries' industrial and agricultural runoff. The aim of this study, which is a part of the ECOTONES project, led by the GIP Seine-Aval, is to utilize a multi-disciplinary approach to assess the health status of a key invertebrate of estuary functioning: the annelid polychaete *Hediste diversicolor*. The approach is based on endpoints at multiple levels of biological organization (cellular, individual, population) so as to estimate the overall health of this species in the Seine estuary. For each biomarker studied (acetylcholinesterase: AChE, catalase: CAT, glutathione *S*-transferase: GST, superoxide dismutase: SOD, thiobarbituric acid reactive substances: TBARS, energy reserves), results of the current campaign (March 2015) in Seine were compared to historical data previously acquired from samples collected in reference sites (Authie and Bourgneuf). *H. diversicolor's* population trends depicted lower biomass and density, smaller individuals with delayed reproductive potential compared to samples from reference sites. Biochemical biomarkers results also indicate that ragworm was being severely impacted at the cellular level exhibiting low levels of energy reserves (glycogen and lipids), of AChE activity (neurotoxicity), and of GST values suggesting defense mechanisms being overwhelmed. The high levels of Catalase and TBARS compared to values of reference sites suggest an oxidative stress endured by organisms. In addition, a predictive model for BAC and EAC determination using a linearized regression model (with "K-means clustering" method) has been developed. **Keywords**: Levels of biological organization, *Hediste diversicolor,* Biomarkers, Background and Environmental Assessment Criteria.

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Adaptive responses of a marine filter-feeder, the common blue mussel, to chronic contamination in a harbour located within urbanized environment.
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Conjugated effects of chronic contamination and temperature increase affecting coastal waters are to be addressed by marine biologists when considering global change in the ocean. Adaptive responses of a marine filter-feeder, the common blue mussel *Mytilus spp*., have been investigated in a pseudo-estuarine area, the Bay of Brest (Brittany, France). Shipping activities in the medium-sized harbor of Brest with both trade and navy port have generated for decades a chronic contamination by POPs. Actual contamination levels have been established in mussel tissues. To assess biological responses to environmental pressure, the activity of several enzymes involved in major physiological functions, including metabolism and biotransformation/detoxication, has been measured along with biometric and overall health parameters. In order to consider the intra-population

variability, several cohorts were recognized based on shell length and further sampled. Considering that two species, *M. edulis* and *M. galloprovincialis*, and their hybrids are sympatric in this geographical area, the genetic status of each individual has been determined by using molecular methods. As a complement to these observations in native individuals, mussels collected at a distant and less contaminated site in the Bay have been caged in the harbor. After one month, contrasted, biochemical responses among groups suggested an adaptive process in native mussel in order to overcome deleterious effects of environmental stress. This set of observations could contribute to a better understanding of how biological responses are induced in the blue mussel which is becoming a major sentinel species in the survey of global changes effects in coastal ecosystems.
Keywords: Coastal ecosystems, anthropogenic pressure, invertebrates, adaptation
This work was supported by a grant from both ANR and NSERC Agencies (joined project France-Canada) as a part of the research project IPOC (Interactions between POllution and Climate changes: Development of improved monitoring strategy, ANR-12-ISV7-0004-01, 2013-2015)

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Multiple stressors influencing seagrass dynamics in a shallow Mediterranean lagoon
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Seagrasses have a wide spatial distribution, form an important component of many coastal ecosystem and provide essential ecosystem services. Seagrass declines have been observed worldwide due to a variety of (multiple) stressors. The shallow Vaccarès lagoon, Camargue, France is an example of a highly important coastal lagoon situated in a Mediterranean river delta and is surrounded by intensive agriculture. *Zostera noltei* is the dominant macrophyte species and suffered two major declines in 1997 and 2008. The first decline was well explained by salinity and turbidity, whereas the second decline could not be explained by these parameters. Other stressors such as chemicals, nutrients or temperature could be explanatory variables for the second declines. The main aim was to understand the influence of multiple stressors on seagrass physiology and population dynamics in a typical Mediterranean shallow lagoon (Vaccarès lagoon) by analysing the biological, environmental and chemical data. Contamination by pesticides and metals was monitored in water and sediments both in the lagoon and in its main tributaries from 2011 to 2014. The recent dynamics of seagrass was quantified across the lagoon using a 55-points sampling design. Three stress biomarkers were measured *in situ*: photosynthetic pigment composition, chlorophyll fluorescence and rhizome sugar content. Biological, environmental and chemical parameters were analysed by co-inertia analysis. GIS was used to interpolate between measured data points. Co-inertia analysis showed a significant relationship between environmental data and seagrass status matrices. Seagrass dynamics were negatively correlated to the concentrations of some herbicides in water (2,4-MCPA and bentazone) and with metals in sediments (arsenic). Rhizome starch content in winter was negatively correlated to those herbicides and to several metals (arsenic, zinc, copper) in water and/or sediments. These results suggest that environmental contamination may be responsible for the decline of seagrass in some parts of the Vaccarès Lagoon. However, complementary investigations, such as longer monitoring and additional toxicity tests, are required to address the causal link between contamination and seagrass decline. Understanding the influence of multiple stressors on seagrass dynamics and thus possible declines is crucial for environmental risk assessment and to preserve these valuable systems with integrated management strategies.

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Influence of environment on the response capacity to thermal stress in the blue mussel using experimental approach
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In the intertidal zone, organisms are permanently exposed to strong environmental gradients and long periods of stressful conditions including hypoxia, temperatures and eutrophication. Temperature variations impact organisms at both biochemical and physiological processes directly, by restricting enzyme activity efficiency or promoting protein catabolism. Mussels of the genus *Mytilus* are one of the most important marine bivalves along the French coast at both economic and ecologic point of view. They colonized a wide range of environments from estuary to open coastal ecosystems and consequently challenged a large variety of fluctuating parameters such as salinity, temperature, tide, anthropic effluent. They are largely used as model species in biomonitoring programs and laboratory exposure studies. The present study aimed to investigate the cellular and transcriptomic response of mussels having been exposed to various levels of chronic chemical contamination (having distinctive environmental history) may respond to an experimental increase of temperature. In these experiments, blue mussels collected at two sites of the Bay of Brest were exposed in the laboratory to graduated warming and cellular and molecular responses relevant for physiological stress assessment were measured. In addition, considering that two mussel species (*M. galloprovincialis*, *M. edulis*) and their hybrids are present in the study area, we investigated possible differential responses in order to identify genetic basis of metabolic responses and further, differential sensivity of species to environmental, thermic stress. Results indicated various pattern of response when considering the different parameters analysed (population origin, temperature effect, tissue, hybrid status). Metabolic depression is observed at the highest temperatures for both populations studied but some differences remains at lowest temperature according to the site origin. More, hybrid mussels show some difference in their response compared to “pure” species *M. galloprovincialis* highliting the importance of hybridism in the species adaptation.

Identification and prioritisation of hazardous pollutants in the aquatic environment - the role of effect-directed analysis, monitoring and modelling (II)

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Evaluation of exposure algorithms used for prioritisation of pharmaceuticals in the environment
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Prioritisation methodologies provide potentially useful tools for identifying which from the plethora of current active pharmaceutical ingredients (APIs) could pose the greatest risk to the natural environment; thus focusing expensive laboratory testing or environmental monitoring towards APIs of greatest concern. The use of risk-based approaches that combine exposure and effect models with environmental measurements has become common practice. However, the reliability of these approaches is still unclear. Therefore, in this study we evaluated the accuracy of exposure algorithms commonly used for API prioritisation. An initial stream water occurrence study was conducted for 97 APIs in the City of York. These APIs covered a broad range of therapeutic classes and physico-chemical properties. Predicted environmental concentrations (PECs), obtained using prioritisation exposure algorithms, were then compared to measured environmental concentrations (MECs) to evaluate the general accuracy of predictions. Initial results reveal that the exposure algorithms for almost half of prescription APIs detected provided PECs that fell below MECs. Conversely, another third had a PEC/MEC ratio greater than 3, an overestimation indicating limited accuracy. A stepwise exclusion of select pharmaceutical fate considerations within the exposure algorithms included dilution, metabolism and wastewater treatment removal to target potential contributors to under- or overestimates of the PECs. Metabolism and wastewater treatment removal terms were identified as the largest source of deviation of PECs from MECs for the 97 APIs under investigation. Follow-up research will include a more detailed fate and transport study over a 12 month period to better understand the prioritisation algorithm components that are the driving factors behind the primary differences between PECs and MECs.

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A Weight of Evidence Approach to the Eco-Risk Classification of Organic Chemicals Using Chemical Profiling of Hazard and Exposure
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Environment Canada and Health Canada have begun the third phase of the Government of Canada’s Chemicals Management Plan (CMP3). Key to the planning of CMP3 was the development of efficient approaches to dealing with the large number of substances remaining to be addressed by 2020 as committed to be the Canadian Government under the SAICM agreement. A large number of organic substances have been addressed using Rapid Screening Techniques that largely focus on low tonnage thresholds (in silicotools and empirical data have been combined to generate substance profiles. A potency profile can include mode of toxic action, tissue reside toxicity ratios, receptor binding (estrogen/androgen), chemical activity, chemical reactivity (covalent interactions), aquatic and terrestrial foodweb hazard assessment factors (HAFs) generated using the Risk Assessment IDentification And Ranking (RAIDAR) model, bioactivity (TOXCAST/Tox21) and bioavailability. An exposure profile includes emission rate, overall persistence (P_{ov}), long-range transport, and the ratio of critical emission to actual emission as determined by the RAIDAR model for an emission to water. The potency and exposure profiles are compared to classification rules to determine the level of concern in the environment. Initial classifications are examined to determine robustness and consistency within a grouping afterwards resulting in final classifications. The approach will be demonstrated using 3 organic substances classified as higher, moderate and lower concern for CMP3.

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Identifying the single most dangerous chemical present in UK surface waters following a risk ranking exercise
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The aim of this project was to identify which of the many chemicals we contaminate the natural environment with are likely to be causing the greatest harm. Whilst this may at first seem obvious, the reality is that environmental science can be pushed in many directions with actual risk not being the sole criterion for the research. In this exercise the focus was on chemicals subject to chronic discharge from the human population, rather than those more associated with spill accidents, or chemicals discharged from a particular industry in a discrete location. The 73 chemicals studied in detail included metals, pharmaceuticals, persistent organic pollutants (POPs), pesticides, biocides, surfactants, plasticisers and nanoparticles. For each class, selected representatives were studied in detail to hopefully act as a guide to the risk of that group as a whole. The principle of the data collection was to be representative of the spread of species and effects reported. Typically around 50-100 effect data points and 50-100 measured data points were used. What stands out from the results is the magnitude of the differences in risks between the chemicals. So for example Zinc based on its toxicity and dissolved river concentration would appear to represent a 10,000-fold greater risk to UK rivers than say nano-silver or propranalol and 100,000-fold greater risk than metoprolol! Using different refinements such as only using recent UK water measurements, dissolved concentrations, lethal or sub-lethal effects and only those compounds with BCFs higher than a 1000 still led to metals such as zinc and copper dominating the top 10 risk.

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Estimating emissions, concentrations and combined effects of REACH substances in EU waters
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In this contribution, we present the analysis of the emissions, exposure and net expected mixture impacts from 4000+ monoconstituent organic chemicals, registered under REACH up til February 2015. A train of models was used to describe, predict and analyze potential impacts of REACH chemicals in aquatic ecosystems. Starting from registered EU tonnages, emission rates to an imaginary downstream local EU-environment consisting of air, water and soil were estimated by means of a newly developed, REACH-based release estimation model which accounts for all major use stages and known usages during the substance Life Cycle. In a subsequent fate modeling step, a simple unit-world fate model, combined an STP model were used to predict steady-state masses in air, water and soil. In a final calculation step, expected impacts of the REACH chemicals to aquatic ecosystems were estimated using SSD-based hybrid toxic pressure calculation. Derived emission rates, expected environmental concentrations and predicted impacts on an imaginary EU freshwater ecosystem were compared with results of water quality monitoring programs, such as the third Joint Danube Survey (JDS3). Total market volume of REACH-registered 'mono constituent organics' amounted to $2x10^8$ ton/yr in 2015. Emission rates vary strongly among usages; it is estimated that typically near 25% of the marketed volume is released at some point during the chemical's life cycle. Predicted exposure concentrations in an imaginary EU fresh water body range from less than 1 fg/L to nearly 1 mg/L. Potentially Affected Fractions of species that result from such exposures range from practically zero as much as 10%. Toxic pressures from individual substances are usually very low. However, occurrence of high toxic pressures from individual chemicals at specific locations, although often highly uncertain, cannot be ruled out. The combined exposure to mixtures of chemicals is expected to result in undesired toxic pressures at many places, particularly so in downstream parts of river catchments. Emission, fate and impact-oriented modeling helps to identify priority mixtures and drivers of mixture toxicity, which are important steps towards the definition of River Basin Specific Pollutants and the revision of priority pollutant lists.

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Spatially and temporally resolved exposure modeling with STREAM-EU: Prediction of environmental concentrations of multiple industrial compounds in European river basins
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The Water Framework Directive (EC, 2000) aimed at achieving good ecological status in European water bodies by 2015. But currently good ecological status occurs in less than half of the European freshwater bodies, with a large proportion of them having an unknown status due to insufficient monitoring. Ecological degradation of water bodies is often caused by pollution from the thousands of commercially available chemicals that are widespread in the environment. Knowledge about the environmental occurrence of these chemicals is in many cases lacking despite being of great relevance. Appropriate estimates of environmental concentrations can be obtained using models. However, existing large-scale models have only been applied to simulate down-the-drain chemicals and are not applicable to some of the substances in use. Within the EU-FP7 project SOLUTIONS the challenge was addressed by developing STREAM-EU (Spatially and Temporally Resolved Exposure Assessment Model for European basins), a large-scale fate and transport model able to simulate spatially and temporally resolved environmental concentrations of any organic substance in all relevant environmental media. In the current work, STREAM-EU was used to simulate environmental concentrations of multiple industrial compounds subject to REACH (EC, 2006) registration in European river basins.\nThe simulated industrial chemicals were selected from priority pollutant lists and cover a broad range of uses, functional groups and toxicological effects. An assessment of the predicted exposures was done by comparing monitored and simulated bulk concentrations using field data from the Joint Danube Survey 3 (JDS3) with a reasonable agreement found between model estimates and measurements.\nOur newly developed model constitutes a large step towards wider understanding and quantification of industrial chemicals' exposure. Its applicability to any type of organic substance, any European catchment and both diffuse and point source pollutants makes it invaluable, for example, to perform large-scale human and ecological risk assessments, evaluate and choose abatement options and prioritize pollutants. All of these model applications, among others, are planned within the SOLUTIONS project.

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Pollutants 2030: Predictions based on developments in society
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Patterns of pollution in river basins change with time. Management strategies should address such developments. Many scenarios are published which describe future changes in society: climate change, demographic change, urbanization and others. Specific trends can be predicted (with uncertainties) already now. Does this help to get a picture on future pollutants? In most of the existing scenarios, impacts on (aquatic) ecosystems are not directly addressed. However, scenarios often describe trends which can be linked to the use of chemicals. Therefore, they can be the starting point to analyse implications on future use and emissions of chemicals and consider how to anticipate in terms of risk prevention.
Sector-specific trends have been analysed with a wide range of experts from the respective sectors with focus on health care, food production and supply, urbanization and changes in land use and new technologies. It could be shown that these developments have many and often complex implications on future pollutants. Patterns of use will change e.g. for pharmaceuticals, biocides, household chemicals as well as for specific groups of industrial chemicals. In many areas legacy chemicals will contribute significantly to the future impact on the environment – besides really unexpected new substances. This refers not only to substances which have been used in society decades ago and are still present in the environment or in "stocks", e.g. buildings, so called legacy chemicals. It refers also to a large number of "new materials", which are often based on the use of already existing chemicals in processes or technologies for which they were not originally conceived. Furthermore, completely new molecules and materials have to be expected with not yet known usage, field of application and properties and transformation products. Some of the future trends can be integrated in exposure and risk modelling. Examples are predictions on demographic change and changes in the consumption pattern of pharmaceuticals during life time. Other trends can have implications for effect monitoring. The expected increase of emissions of pharmaceuticals could stimulate the development of drug-specific monitoring endpoints, e.g. behavioural changes in fish. These findings will be used in the project SOLUTIONS to develop prioritization and risk reduction approaches for individual chemicals and mixtures and for assessment of abatement and policy options

Identifying and regulating PBT and vPvB chemicals: Requirements,challenges and policy implications

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Comparison of P, B and T properties of parasiticides and harmonisation of the basis for an environmental assessment at the EU level
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Persistent, bioaccumulative and toxic (PBT) substances are of specific concern and their identification is part of various regulations. The environmental risk assessment of veterinary pharmaceuticals includes a PBT screening and, if required, a PBT assessment for the active substances. However, so far it is not clear how PBT properties should be considered in the risk/benefit evaluation of veterinary pharmaceuticals, and which consequences the classification of an active substance as PBT might have. Furthermore, there is no complete and consistent database for a comparative assessment of P, B and T properties of different parasiticides. The present project contributes to filling these data gaps by deriving octanol/water partition coefficients and fish bioconcentration factors for selected parasiticides, and by compiling available data on the excretion of parasiticides by pasture animals and on their effects on dung organisms. Octanol/water partition coefficients (P_{ow}) for ivermectin (log P_{ow}: 5.6) and selamectin (log P_{ow}: 6.0) as determined with the slow-stirring method (OECD test guideline 123) are higher than previously available values. Bioconcentration of ivermectin in zebrafish (*Danio rerio*) is currently investigated according to OECD test guideline 305. First results indicate that a steady state is reached relatively fast. In a second test, bioconcentration of doramectin will be studied. Based on the evaluated literature data the toxicity of parasiticides to dung organisms can be classified as follows: doramectin > ivermectin ≈ eprinomectin > moxidectin. The obtained results will be used to identify effective and feasible risk management measures for parasiticides with PBT properties that will be discussed with competent authorities, veterinarians and farmers.

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The Origin and Evolution of Persistence Criteria for PBT Chemicals and Persistent Organic Pollutants
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Public concern over the effects of persistent chemicals began in the early 1960s. Since then, significant scientific advances have increased our understanding of persistent, bioacumulative, and toxic (PBT) chemicals and the properties and processes that influence their fates in, and adverse effects on, humans and the environment. In addition to the scientific advances, a number of legal instruments and agreements for global, international, and national identification and control of persistent organic pollutants (POP) and PBT chemicals have been adopted. However, some of the rationales and thoughts that were relied upon when the first criteria were developed to identify and categorize PBT chemicals and POPs have not been carried forward. Criteria for identification of persistent chemicals are single-media half-lives based upon available data for neutral hydrophobic “reference chemicals”, derived under laboratory conditions, and consensus-based policy decisions. Criteria have evolved over the last decades due to the diversification of the protection aims under various national regulatory frameworks and international agreements, advances in methods for estimation of physical/chemical properties, and the identification of chemicals which are non-traditional POPs. The numerical criteria serve as ‘yardsticks’ for assessing persistence properties of POPs and PBTs. From a scientific perspective, it is logical to use the same reference conditions as were defined for setting criteria values, when assessing a substance under review. If conditions, e.g. temperature or organic matter content, are changed for the determination of the properties of a substance undergoing assessment, the comparison may no longer apply. A better understanding of the robustness of persistence indicators, in particular for water-sediment systems, and a consensus on the interpretation of non-extractable residues in soil and sediment, would improve the persistence criteria for screening and classification. Setting appropriate numerical criteria for P_{ov} would ensure that environmental contamination is reduced in a reasonable time frame. Weight of evidence should be applied in developing these revisions. Matthies M, Solomon K, Vighi M, Gilman A, Tarazona J. The Origin and Evolution of Assessment Criteria for Persistent, Bioaccumulative and Toxic (PBT) Chemicals and Persistent Organic Pollutants. Integrated Environmental Assessment and Management (accepted).

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Use of monitoring data to assess PBT, vPvB and POP properties of chemicals
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Environmental risk assessments regarding PBT, vPvB and POP properties of chemical substances are mainly based on modelling and laboratory data. However, both the current ECHA guidance and the Stockholm Convention include the additional use of monitoring data to assess these properties. For instance, according to the ECHA guidance findings of significant concentrations of a substance in remote and pristine environments may provide evidence of long-range transport potential (LRTP) and high persistence. Significant concentrations of a chemical in higher levels of the food chain in unpolluted areas are also regarded as an indicator of high persistence besides bioaccumulation. In the Stockholm Convention monitoring data from remote regions, esp. detection in the Arctic/Antarctic, are regarded as crucial information on the LRTP of a chemical. If sufficient data on production and usage volumes, and/or emissions of a substance are available, a more comprehensive assessment is possible including, e.g. temporal trends of contaminations. In a recent project the options of using monitoring data to assess persistence, bioaccumulation and LRTP of 6 (potential) PBT substances were tested. Data were compiled from monitoring programmes operated in Germany or with German participation and supplemented by monitoring data from other European countries and the Arctic Monitoring and Assessment Programme. In a second project, detection of substances in the Arctic/Antarctic was used to identify potential POP candidates in the context of the Stockholm Convention. Monitoring data and other relevant information were also compiled to confirm the assessment of new POP candidates, which had been prioritised based on modelling data. The results show that, besides prioritisation based on modelling data, detection of substances in remote regions can be used to identify potential POP candidates. For both approaches additional data are required to confirm substance prioritisation. Furthermore, monitoring data are an essential information to support PBT or POP assessments based on modelling and laboratory data. This is especially true for LRTP for which laboratory data are hardly available. Appropriate monitoring data tailored to specific issues referring to persistence, bioaccumulation and biomagnification in food webs are currently accessible for few substances only. Thus, such monitoring studies are esp. recommended if modelling and laboratory studies yielded insufficient results.

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Screening and prioritization of chemicals for PBT behavior: state of the art on the consensus approach by PBT Index and PBT Profiler
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The limited availability of comprehensive data to describe Persistence, Bioaccumulation and Toxicity (PBT) properties pose a serious hindrance to the categorization of chemicals as PBTs or vPvBs, which use is regulated in REACH through authorization and additional plans for safer alternatives. A possible solution to this problem is the use of *in silico* approaches, such as those based on QSARs, which are valid alternatives to animal testing. These approaches generate predicted data and help in early identification of chemicals of high concern and in the definition of priority lists. In the context of screening and prioritization tools for PBT-assessment, we proposed a screening method founded on a consensus approach between two different QSAR-based models for the identification of PBTs. We developed this approach by combining predictions generated by the Insubria PBT Index model, implemented in the QSARINS software (which we developed at University of Insubria), with those calculated by the US EPA PBT Profiler, a freely available online tool for the preliminary PBT screening of organic chemicals. Predictions which were in agreement between the two models were more consistent, reliable and with a higher probability to correctly identify PBT chemicals, than those generated by one of the two models taken singularly. During the last years, we have applied this consensus approach to screen and prioritize big datasets of chemicals of environmental concern with heterogeneous molecular structure, and to screen specific groups of chemicals such as Flame Retardants, Personal Care Products and Pharmaceuticals. The good agreement between the two models, supports the utility of the consensus approach to highlight the need for urgent experimental tests on compounds prioritized in agreement as PBTs. The screening case sudies, described in this presentation, are examples of how results generated by the Insubria PBT Index and by the US-EPA PBT profiler, which are both based on chemical structure, can be combined in order to identify the most dangerous among the studied compounds. It is evident that the reliability of the predictions is higher for compounds that are predicted in agreement by different methods. The consensus approach combines the perspectives given by multiple modelling methods, which are based on different structural features and mathematical approaches, and is useful to integrate PBT/vPvB assessment procedures based on thresholds criteria.

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Regulatory PBT/vPvB assessment of UVCB-substances
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The experience in assessment of PBT/vPvB properties of substances of Unknown or Variable Composition or Biological origin (UVCB-substances or UVCBs) has increased considerably in recent years. During the last four years alone over 50 UVCB-substances were assessed for their PBT/vPvB properties by REACH competent authorities in Europe. Furthermore, a very high number of UVCBs have been assessed by REACH registrants so far. Generally, it is necessary to assess the PBT properties of all relevant constituents of a substance. However, due to their complexity and variable nature, accurate assessment and testing of all individual UVCB constituents is often not technically feasible or resource and cost-effective. Efficient assessment and testing strategies are therefore essential. The following pragmatic approaches have been applied to date: (1) Whole substance approach; (2) Fraction profiling approaches; (3) Known worst case constituent approach; (4) a combination of one or more of these three approaches. This presentation explains the approaches and gives an overview of their application with a few examples : MCCPs, styrenated phenol, coal tar pitch, high temperature, polybrominated diphenyl ethers and *tert*-dodecylmercaptan.

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How to apply socio-economic analysis to REACH-authorisations of PBT and vPvB chemicals? A critical synopsis of approaches and decision-criteria
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A core aim of the European chemicals legislation REACH is to ensure that the risks caused by substances of very high concern (SVHC) are properly controlled. Authorisation – i.e. the formal approval of certain uses of SVHC for a limited time - is a key regulatory instrument in order to achieve this goal. For persistent, bioaccumulative and toxic (PBT) or very persistent and very bioaccumulative (vPvB) SVHC listed on Annex XIV of REACH, decision-making on the authorisation is conditional on a socio-economic analysis (SEA), in which companies must demonstrate that the gains from keeping a chemical in use outweigh expected damage costs for society. The current setup of the REACH authorisation process, however, raises a number of questions concerning the practical applicability of this tool. We suggest a tiered framework for which we discuss key analytic steps and informational requirements which we consider crucial in order to operationalise SEA of PBT and vPvB chemicals. Using the case of hexabromocyclododecane (HBCDD), an Annex XIV PBT substance which had a sunset (phase-out) date in August 2015, we investigate key challenges that need to be met in order to ensure that a SEA can be operationalised in a meaningful way. We illustrate that, if monetary estimates for health and environmental impacts are available (e.g. from impact-specific valuation studies), a SEA will deliver the optimal period of use of a chemical. Thus, an authorisation should be granted with a review period that extends until marginal discounted benefits of use equal marginal discounted damage costs. A crucial aspect of this path is the selection of a concern-based discount rate. Another challenge is to examine the use of environmental monitoring data for determining media-specific impact functions. However, a monetary impact valuation is unlikely to be feasible for most PBT chemicals. Still, if sufficiently reliable information on a chemicals' toxicity is available, non-monetary evaluation approaches, for example multi-criteria or cost-effectiveness analysis, can be applied in a SEA. For vPvB chemicals, in particular, a SEA applying (explicitly or implicitly) efficiency decision-criteria turns out to be inappropriate because of deep uncertainty regarding vPvB's toxicity and risks. We therefore suggest to adopt an alternative authorisation route for vPvB chemicals, which should be based on robustness criteria such as, for example, the 'minimisation of maximum regret'.

How can we improve the link between academic research and policy-making in order to advance chemical risk assessment and management?

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CRED - Criteria for Reporting and Evaluating ecotoxicity Data
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The derivation of safe concentrations of chemicals for ecosystems, such as Predicted No Effect Concentrations (PNECs) or environmental quality standards (EQSs), is an important aspect in a large number of (legal) frameworks worldwide. Different datasets are used in the various frameworks, and combined with the use of different assessment methodologies this often leads to the derivation of different PNECs/EQSs for the same compound. However, even when the same set of study reports and/or references from the open literature is available and the same guidance documents are used, there is no guarantee that risk assessors will arrive at the same list of usable endpoints from these studies. The evaluation of scientific reliability and relevance of ecotoxicity studies is often subject to expert judgment, and results may differ between frameworks and between assessors within one framework. To improve the evaluation of aquatic toxicity studies, the CRED project was started. CRED stands for Criteria for Reporting and Evaluating ecotoxicity Data. The aim of CRED was to improve the reproducibility, transparency and consistency of reliability and relevance evaluations amongst frameworks, countries, institutes and individual assessors. We also aim to improve the usability of peer-reviewed literature for substance evaluations, and in the end improve the exchange of assessment results between frameworks. We will present the CRED method, which includes a set of 20 reliability and 13 relevance criteria with an extensive guidance, as well as a guidance on how to report data from ecotoxicity studies. To test the applicability of the methodology, we performed a ring test with over 80 risk assessors from Europe, Asia and North America, representing academia, regulatory agencies, consultancies and industry. Ring test participants evaluated the CRED evaluation method to be more accurate, applicable, consistent and transparent than the often-used Klimisch method. The CRED evaluation method is accompanied by reporting recommendations for aquatic ecotoxicity studies, with 50 specific criteria divided into six categories: general information, test design, test substance, test organism, exposure conditions, and statistical design and biological response. An ecotoxicity study in which all important information is reported is more likely to be considered for regulatory use, and proper reporting will support the peer-review process.

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Lessons learned from a multi-country project under the SAICM Quick Start Programme
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Under the SAICM Quick Start Programme, a multi-country project on "Training on risk assessment for chemicals at national level in a global context" was implemented by the governments of Armenia, Chile, and Ghana in the period of 2010–2014. The International Panel on Chemical Pollution, IPCP, was the executing agency of the project. The overall aim of the project was to make available, in each of the three countries, a set of methods, tools and data for chemical risk assessment ("materials") and to conduct workshops and national training activities that enable scientists, government representatives and other stakeholders to access and use these materials. In the initial phase of the project, a kickoff meeting with representatives of all countries, IPCP scientists, and experts from international organizations (WHO, OECD) as well as national workshops in each of the countries were conducted. This was followed by a phase of national case-study work (case-study topics were PCBs and organochlorine pesticides in Armenia, heavy metals in Chile, atrazine in Ghana) in collaboration with IPCP scientists and international experts. Finally, the results obtained were collected, discussed and compared at a synthesis workshop at the end of the project. The project required coordination of partners from several different types of institutions: government institutions in each of the countries; a range of national stakeholders such as national research institutes, NGOs, universities, and industry associations; experts from international organizations, IPCP scientists, and the SAICM secretariat. Important conclusions from the project are: (i) Methods for first approximations or estimates of the components of a chemical risk assessment, such as emission rates of a chemical, were found to be useful and of immediate benefit. (ii) Approaches that enable project participants to establish a "big-picture" understanding are important because often there is an extensive body of data from various projects available in a country. Offering a framework that can accommodate existing data helps reconcile existing data and supports long-term thinking and collaboration of experts from different fields. (iii) More time than anticipated may be needed because local stakeholders, interest groups, and societal actors need to be involved; it is not easy to estimate in advance how much time will be needed for this process and what the outcomes of the local stakeholder discussions will be.

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Using the ecosystem services framework to link scientific research and policy-making: a case study of Lake Tai, China
Y. Pan, University of Sheffield / Animal and Plant Sciences; L. Maltby, The University of Sheffield / Dpt of Animal Plant Sciences
One way of linking scientific research and policy-making is to increase public participation into decision making processes, because public perceptions can play a significant role in identifying protection goals for use in chemical risk assessments. One approach to forming protection goals is to identify ecosystem services (i.e. benefits that we obtain from ecosystems) valued by society. Different sections of society may not have the same ecosystem values and this should be considered during the establishment of protection goals. Clear protection goals are vital for many developing countries with rising economies, such as China, which suffer from unsustainable development and environmental degradation. The aim of this study was to investigate the value of ecosystem services to local communities using Lake Tai, China, as a case study. Lake Tai is the third largest

freshwater lake in China and is affected by severe anthropogenic threats, including chemical pollution that can negatively impact ecosystem services. Four cities surrounding the lake were investigated by conducting interviews with 255 rural and 252 urban respondents. There are similarities and differences between the value of services across cities, as well as across rural and urban communities. Each group of respondents ranked freshwater provision as the most important service but other services were valued differently between respondents situated at different locations around the lake. Rural communities ranked directly-used services (i.e. food provision and flood regulation) higher than urban communities, who ranked indirectly-used services (i.e. cultural services) higher. Prioritised services can be adversely impacted by chemical pollution in Lake Tai and 40% of the respondents were concerned about water pollution. By identifying services prioritised by the public, we can then identify drivers that support the services and specify protection goals. This study sets out a framework for investigating the ecosystem values of different communities. In addition, it highlights the question of how protection goals should be set when different sections of society have varying ecosystem values. By using the ecosystem services framework, this study has linked scientific research, social sciences and policy-making.

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Use of the SEA and DSFA in risk assessment and management for chemicals of concern

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To alarm inapprehensive upcoming concerns so as to avoid them beforehand is granted as the ultimate goal of risk management and communication. This goal necessitates comprehensive, systematic appraisal of possible adverse impacts with a holistic consideration of social, economic and environmental impacts on a long-term temporal scale before decision making. Here we propose that a cost-effectiveness-oriented socio-economic assessment (SEA) framework, coupling with a dynamic substance flow analysis (DSFA), is competent for such appraisal in national management of hazardous chemicals to implement multilateral environmental agreements. We exemplified the application of the two approaches using the case of phasing out hexabromocyclododecane (HBCDD) in China. The SEA results indicate that the schedule of phasing out HBCDD production and new uses within two five-year exemption periods generates higher environmental and health benefits while that within a five-year exemption period leads to smaller economic and social costs. A decision can be biased if we ignore the environmental and health benefits and their related positive externalities. The DSFA results show that a whole century or longer time will be needed to eradicate HBCDD from the present in-use and waste stocks although production and new uses will stop soon. Therefore, future management efforts should be devoted to end-of-life disposal management. Our case highlights the implication and importance of the SEA and DSFA in national chemical management and risk communication.

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Participative valuation of ecosystem services of aquatic ecosystems: Evrotas river basin Greece

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Little is known beyond the described effects of single stressors on the chemical and ecological status of water bodies and on their ecosystem functionality. This lack of knowledge limits our capacity to understand ecosystem responses to multiple stressors and to define a programme of measures that can improve the ecological status of a water body as sought by the European Water Framework Directive. The ecosystem services framework aims to support informed decision making by explicitly linking the goods and services produced by functioning ecosystems to human well-being illustrating the broad impacts of various land-use scenarios. The Economics of Ecosystems and Biodiversity (TEEB) approach provides a framework for assessing multiple stressor and multiple outputs of a river basin, facilitating management of a complex system. Economic valuation includes monetary and non-monetary methods. Monetary methods although under continuous development and construction are well documented [3], [4], [2]. Non-monetary valuation relies on perception and values of stakeholder at large. The main research question addressed is: How do stakeholders perceive Ecosystem services and functioning at river basing level? The practical framework to elicit stakeholder knowledge and to enable discussion around the functioning of Ecosystem services is based on a participative workshop with representatives from the public and private sector involved in water management, nature management, cultural heritage and water related economic activities, municipal, and regional planning. A brief presentation of Ecosystem definition and types was given in order to provide the participant with the same conceptual framework and basic knowledge of TEEB. Group-dynamic was rhythmed by group exercises, restitution of group findings in plenary session enabling expression of view-points and social learning. The core activity of the workshops is co-construction of ecosystem services, in three steps: (i) identification of ecosystem services in groups of four to six participants, (ii) plenary exchange of the findings, in order to identify the major ecosystem services, made specific for the river basin and (iii) individual ranking of ecosystem services by participants. The case study is the Evrotas river basin in Greece, 25 valid questionnaires were collected and analysed.

Challenges in data analysis, weighting, valuation and visualisation - How to enable decision makers to make trade-offs while being transparent for all stakeholders

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Combining Fuzzy sets with LCA to weight global environmental impacts with local limits of ecosystem capacity

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Small hydropower plants (SHP) are discussed very controversially. They are essential for the realization of the Swiss Energy Strategy 2050 and looking at the impacts like climate change, energy demand or emissions SHP belong to the most eco-friendly power plants. However they can have relevant impacts on flora, fauna and the local ecosystems and these impacts are specific to each location. An overall weighting taking into account the relevant impacts and local aspects of ecosystems capacity is crucial for a decision support. The goal of this project is to build an evaluation tool for SHP, taking into account all the different environmental impacts, local tipping points and economic aspects. So the challenges are: To combine quantitative indicators e.g. from LCA with qualitative criteria on the local ecosystems. Taking into account the limits and capacities of the local ecosystems. Expressing the expert knowledge on the relation of different factors and ecosystem capacity even if they are controversy. To get a cost efficient method to be applied at SHP projects with limited budgets. After discussions with a variety of experts and studied different evaluation methods it become clear that for modelling expert knowledge given in quantitative as well as in linguistic terms the best method is fuzzy set theory (FST). This method gives the possibility to weight the different LCA results, to introduce qualitative expert judgment and furthermore different even contradictory judgments to be taken into account in the same model. A group of experts from different fields like hydrologist, biologists, from NGOs, administrations, technicians and LCA specialists has been formed to build the model. The first evaluation has shown promising results but further improvements are necessary. The experience from evaluation and modelling has shown that this method is very suitable to evaluate complex systems like ecosystems, taking also into account their capacity. The important advantages of this method are: representation of qualitative expert knowledge with linguistic terms bringing together in a mathematically exact way quantitative and qualitative data and knowledge nonlinear relations can be handled and FST makes the interpolation floating transitions which are typical for environment can be handled FST can give more than one 'right' result typical for human reasoning. Doing all this in a transparent way.

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Reversibility assessment: how to improve the weighting of impacts in LCA

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Weighting impact categories in LCA is an optional step according to ISO14040. However, in the context of LCA in support to policy, weighting may help in the: i) identification of the most relevant impact categories, i.e. to define Product Category Rules (PCRs) for product labelling (as in the case of Product Environmental Footprint – PEF – of products (EC, 2013); ii) to address eco-innovation policies and strategies towards the most effective solutions for decoupling impacts from production; iii) presenting results as a single score. Currently, the ILCD recommandations for life cycle assessment (EC-JRC, 2011) as used in the product environmental footprint (PEF) (EC, 2013) entail 15 impact categories at midpoint. The identification of the most important impact categories at the moment is done adopting a 1:1:1: weighting. Over the years, several weighting set has been proposed in literature (see a recent overview in Itsubo, 2015) such as: proxy methods, midpoint or endpoint methods following different approaches such as e.g. distance to target, monetisation, panel- based sets etc. However, all those methods present stenghts and limitations among which the capability of assessing the environmental relevance of the impacts from a sustainability point of view. The present work aim at discussing to which extent sustainability principles, such as the avoidance of irreversible impacts (see e.g. NRC, 1999), could be a criteria for determining the relative weight of an impact category. Some qualitative attempts have been made (e.g. Soares et al 2006, including the topic amongst criteria for weighting), proposing to a panel of expert to judge the degree of reversibility with a 5-levels score systems spanning from: a natural istantaneus reversibility, to a soley artificial up to irreversible. Moreover, Fanai and Burn 1997 listed specified characteristics of some impacts and direct measures of the severity of other impacts to be used in a “distance metric” formulation to evaluate a reversibility index. A case study on the reversibility of lake eutrophication is reported to discuss potential and limitation in using reversibility as a criteria for weighting. The results stem from the analysis of the evolution of the state of a lake over time, at decreasing environmental pressure in term of human induced nutrient’s loads.

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LCA single score results about PCB-contaminated sediment disposal options lead to reassessment of authorities' decision

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The Klingnau reservoirs main problem today is the silting of the lake. Dredging is being considered to extract the sediments. The dredged sediment can be disposed of by resuspension into the river or by disposal in a land fill. Article 40 states that resuspension can be considered as an option if the flora and fauna downstream is not negatively affected. As the sediment of the Klingnau reservoir is contaminated with pollutants such as heavy metals and PCBs, a qualitative environmental impact assessment commissioned by the authorities was carried out. The concentrations were below the maximum permissible values and indicated that no negative effects were expected by resuspending the sediments. The assessment showed on the other hand that the emissions of the disposal to a land fill option would exceed the maximum permissible values of the Swiss "Clean Air Act for Construction Transports". The environmental impact assessment concluded that the resuspension of the sediments to the river has a lower environmental impact than their disposal to a land fill. In order to verify the results of the environmental impact assessment, the two options were analysed by Life Cycle Assessment. The LCA shows very clearly that the disposal of the dredged sediment by resuspension into the river has a much higher environmental impact than the disposal in a landfill. This is mainly due to the PCB and heavy metal contents in the sediment which in case of the resuspension option affects the river ecosystems negatively. This example shows how important it is to perform a comprehensive assessment in order to evaluate the environmental consequences. The consequence of the LCA results for the Klingnau reservoir dredging project was that the planned resuspension of the dredged sediments to the river was discarded and the concept is currently being revised. As it has been shown in this paper it can prevent severe misjudgements compared to decisions that are only based on legal limits for single pollutant emissions. The presentation of single score LCA results plays an important role in the process of persuading decision makers to change their mind for an environmentally preferable option as such results usually are clear and easily understandable because they don't need further interpretation.

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How far can changes in consumer behaviour take us on the path to environmental sustainability?

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It has recently been proposed to normalise environmental impacts according to environmental carrying capacity in LCA, to arrive at normalised indicator scores expressed as occupations of carrying capacity. This can facilitate the evaluation of whether a studied anthropogenic system is environmentally sustainable. This study presents the first application of carrying capacity based normalisation to a real-life comprehensive case study. The case study aims to covers the total annual consumption of goods and services of 1283 urban Danes, based on their response to a lifestyle survey. The goal of this study is to demonstrate the applicability of carrying capacity based normalisation by exploring the extent to which Danes can become environmentally sustainable by practical changes in behavior. The LCIs of total personal consumption were compiled by combining process-based inventory modelling (e.g. for consumption of food and residential heat) with environmentally extended input-output analysis (e.g. for expenditures on electronics and clothing) and extrapolations for other impact categories than climate change. A "minimum impact"-profile was constructed by combining the environmentally soundest qualitative and quantitative consumption behaviour observed in the respondent pool across consumption categories, while disregarding potentially erroneous outliers having unrealistically low consumption values. The average respondent was found to exceed his or her fair share of carrying capacity for five impact categories. For climate change and photochemical ozone formation the exceedance was above a factor of 10. According to the results, the average respondent would be able to decrease his or her impact by 25-50% if he or she adopted the behaviour of the minimum impact-profile. Yet, the minimum impact-profile was found to be environmentally unsustainable for five impact categories, albeit less so than the average profile. The case study results indicate that (quite large) behavioural changes alone are insufficient for achieving the goal of personal environmental sustainability for all LCA impact categories in a Danish setting and must be supplemented by policies targeting, for example, eco-efficiency improvements of the energy system and public services. The application of carrying capacity based normalisation references in this study demonstrates that LCA, in principle, can be expanded from answering "which is better?" to also answering "is better good enough?"

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Incorporating Lifecycle Thinking in Nanotechnology Risk Control and Sustainability Assessment: The Case of Sustainable Nanotechnology Decision Support System

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Early identification of risks and impacts of engineered nanomaterials over the lifecycle can enable stakeholders to minimize these concerns at an early stage of nano-enabled product development. The SUN Decision Support (SUNDS) system integrates tools for evaluation of the environmental, economic and social impacts of nano-enabled products, and supporting their development toward safety and sustainability. SUNDS conceptual decision framework comprises of two tiers of varying analytical complexity and data requirements. Both tiers utilize Life Cycle Thinking (LCT) and organize the most outputs of individual modules and sub-modules in terms of hotspots to be addressed through remedial measures for risk management and sustainability assessment. The first tier of SUNDS comprises of LICARA NanoSCAN, provides a semi-quantitative evaluation of the environmental, social and economic benefits and the ecological, occupational and consumer health risks of nano-enabled products from lifecycle perspective in comparison to conventional products with similar uses and functionality. SUNDS Tier 2 implements an integrated Risk Control (RC) and Socioeconomic Assessment (SEA) module, in which the RC module comprises three risk sub-modules (Ecological Risk Assessment (ERA), Public Health Risk Assessment (pHHRA) and Occupational and Consumer Human Health Risk Assessment (oHHRA and cHHRA respectively), and SEA comprises all the sub-modules (ERA and HHRA, Life Cycle Impact Assessment (LCIA), Economic Assessment (EA), Social Impact Assessment (SIA)) . Within both RC and SEA modules in SUNDS Tier 2, most of the sub-module outputs are organized within four lifecycle stages: Synthesis, Production, Use and End of Life. RC module pinpoints the hotspots for risks through the lifecycle of nano-enabled products and suggests Technological Alternatives and Risk Management Measures to appropriately reduce these risks. SEA module pinpoints hotspots for TBL impacts based on technical thresholds and user preference profiles through the lifecycle of nano-enabled products. A comparison of the number of hotspots within the lifecycle stages will pinpoint the most affected stage(s) and guide the decision-maker on where to focus remedial action. The proposed abstract discusses the benefits and methodological challenges in incorporating LCT to SUNDS conceptual decision framework, as well as insights gleaned from its application to case studies.

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Harmonised decision-support and guidance in the European SPIRE programme to achieve overarching sustainability targets?

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The SPIRE (Sustainable Process Industry through Resource and energy Efficiency) programme is a public-private partnership brought to life by the European Commission in the EU's research and innovation programme Horizon 2020. A financial contribution from the European Union budget of EUR 900 million is foreseen over a seven year period in order to engage eight sectors of the European process industry: chemicals, cement, ceramics, minerals, steel, non-ferrous metals, industrial water and process engineering in the development of novel technologies for improved resource and energy efficiency. By means of this measure, the ambitious SPIRE initiative (being part of the Europe 2020 strategy) aims to reduce the energy consumption in the European process industry by 30% and the utilisation of primary (non-renewable) raw materials by 20%, compared with the period of 2008 - 2011. The European project MEASURE, a coordination and supporting action within the SPIRE framework, will provide a roadmap for sustainability assessment in the European process industries and directly linked European collaborative projects, since a number of open issues (e.g. in communication, data standards, agreement on sustainability assessment methods) and R&D needs to be dealt with, before such ambitious overarching visions such as the SPIRE sustainability targets can be brought into practice in an efficient, target-oriented manner. Among the key messages of the roadmap is a broader utilization of the stage-gating approach and established decision support and project management methodologies in European funded SPIRE projects. Within a given team, the stage-gating approach could be highly effectively used as a project planning and monitoring tool, to ensure successful delivery of the key objectives against which project success will be evaluated. Consequently, the stage-gating approach should be used pragmatically to stop research activities that are less likely to contribute to the goals of the project within its lifetime. However, this requires some degree of flexibility in funding *within* the project and access to other sources of funding by research groups. To aid the pragmatic use of stage-gating, existing multi-criteria decision methodologies should be integrated into the development process to aid consistent and transparent decision making.

Poster Abstracts

Contaminants of Emerging Concern in the Environment and their Management (P)

MO001
Vertical distribution of legacy and current used pesticides in marine interstitial water from eight Iberian Mediterranean areas
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Organochlorinated and current used pesticides (CUPs: organophosphorus, triazines and others) were determined in interstitial water of sediment cores from eight Iberian Mediterranean coastal areas (Barcelona, Tarragona, Ebro Delta, Valencia, Castellón, Cartagena, Almería and Málaga). Three sediment cores were taken at three different sampling sites per area by using a box corer at bottom depths higher than 50 m. Cores were cut into 1-cm-thick sections between 0 and 18-cm depth, interstitial water was obtained by centrifugation and samples from every site and depth were pooled. Pesticides were analyzed in interstitial water by stir bar sorptive extraction coupled to gas chromatography with mass spectrometry. Triazines, organochlorinated pesticides, organophosphorus and other pesticides were found in interstitial water of the study areas. The total number of pesticides found per each area varied from 6 to 17 compounds. Procymidone and propyzamide were found in all areas at concentrations below 100 ng L^{-1}. Other commonly detected compounds were a-endosulfan, m-parathion, alachlor, chlortal dimethyl and simetryn. Overall the concentrations of CUPs decreased with depth in the considered areas. However, the presence of some legacy pesticides, such as p,p'-DDE was mostly detected in deeper layers.
Acknowledgements This work was supported by the Spanish Ministry of Environment (2-ESMARME and 2-2-ESMARME projects), the Spanish Inter-Ministerial Science and Technology Commission through the 'IMPACTA' (CICYT, CTM2013-48194-C3-1-R) project and the European Union through the European Regional Development Fund (ERDF).

MO002
On-site large-volume solid-phase extraction device - A solution for sufficient sample volumes in comprehensive toxicological and chemical analysis of wastewater effluent
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Recently, more comprehensive approaches such as multi-target, non-target and bioanalytical techniques and combinations of these, have risen next to the more traditional way of monitoring hazardous compounds in wastewater effluents. However, one big issue related to extensive ecotoxicological profiling and chemical analysis is the amount of sample needed for such analysis. In addition to volume, sufficient concentration factors are needed because many of the compounds causing harmful effects are present at very low concentrations (ng/L). The main objective of this study was to apply and test the suitability of a recently developed onsite large-volume SPE device (LVSPE50) to achieve sufficient sample volumes for the comprehensive toxicological and chemical analysis of wastewater effluent. The performance of the device was evaluated by determining recoveries for selected compounds of perfluorinated alkyl acids (PFAS), hormones and bisphenol-A (BPA) from spiked artificial and real wastewater effluent by liquid chromatography mass spectrometry (LC-MS/MS). The LVSPE50 device is based on a system where the water is pumped through multiple steps through the machine (pre-filtration, sampling chamber, SPE cartridge). The device performed well with influent and effluent samples and with some WWTPs even more than 40L of sample was collected and extracted. The recoveries for PFAS were between 1-96 % and 35-109% for hormones and BPA in artificial effluent depending on the compound. Large extraction volumes made it possible to analyze the samples with multiple bioassays (FET, ER-CALUX, AR-CALUX, p53-CALUX, NRR-assay, umu-test, EROD assay) and chemical analysis of more than 400 target compounds (LC-MS/MS).

MO003
Gene expression profiling of bacteria for environmental monitoring
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Major water pollution includes the collective discharge of domestic and industrial waste together with human bacterial pathogens into the environment. Wastewater treatment plants (WWTPs) do not completely remove all the pharmaceutical and organic contaminants, which subsequently end up in environmental waters. A major concern is that contaminants contribute to increased virulence and/or antibiotic resistance of human bacterial pathogens and thus pose a health risk to the community. We have designed and applied a gene expression system for *Pseudomonas aeruginosa* to predict the potential impact of soluble components released from sewage sludge on bacteria. *P. aeruginosa* was selected since it is both an environmental bacterium with bioremediation capacity and an opportunistic pathogen. Thirty-nine selected genes associated with stress response, virulence and antibiotic resistance were analysed by quantitative reverse transcriptase - PCR in bacteria treated with either antibiotics or heat shock for validation or sewage sludge leachates from WWTPs servicing 3 different cities (Eskilstuna, Västerås and Örebro) in Sweden. The molecular profiling of the sludge leachates from WWTPs was assessed using the principle component analysis. The sludge leachates from Eskilstuna affected a greater number of *P. aeruginosa* genes, responsible for general stress response and virulence, while Västerås sludge leachate affected fewer genes and clustered more closely to the control. Örebro sludge leachate had intermediate influence on the gene expression in *Pseudomonas*. This suggests that the presented strategy of *P. aeruginosa* toxicogenimics has potential applications in evaluating the effects of soluble contaminants on bacterial pathogens in environmental waters for considerations in risk assessment.

MO004
Pyrethroid occurrence and distribution in Brazilian fish tissues
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Pyrethroids are organic pollutants with high hydrophobicity used as insecticides. Concern exists about aquatic organisms' exposure to their toxicity. They produce a neurotoxic effect by altering the sodium channels of the nerve cells. Their LC_{50} values can be as low as 0.2 μg/l for aquatic macroarthropods considering a 4-day exposure, and 1 μg/l for fish. They were believed to be excreted or converted to non-toxic metabolites. But a recent work has detected them in wild river fish for the first time at significant levels in 100 % of the samples [1]. Our study investigates the occurrence of pyrethroid compounds in fish from three locations in Rio de Janeiro (Brazil), as the first attempt to determine the occurrence and distribution of pyrethroids in marine fish tissues from Brazil. This is the first study to report pyrethroid levels in wild fish tissues from the sea. Samples were collected from Rodrigo de Freitas lagoon, Ipiranga bay and Itaipu in 2009-2010, including different tissues—muscle, liver and gills—from 20 fish including 10 males and 10 females. The analytical method monitored 10 different pyrethroids. For the sample preparation lyophilized sample was spiked with internal standards, extracted by sonication and underwent a clean-up with alumina and C18 SPE cartridges. Extracts were analysed by GC-NCI-MS/MS. Method recoveries ranged 53-116 % and method LODs and LOQs were 0.02-0.46 ng/g lipid weight (lw) and 0.08-1.54 ng/g lw, respectively. Pyrethroids were detected in all the fish and most tissues. Total concentrations were in the low limit of the range found in the wild fish from Spanish rivers (12-1,508 ng/g lw) and those found in dolphins from Brazil (7.0-68 ng/g lw) [1,2]. Half of the selected analytes were detected; cypermethrin and permethrin being the main contributors to the pyrethroid profiles. *Acknowledgments* – This work has been funded by the Generalitat de Catalunya (Consolidated Research Group Water and Soil Quality Unit 2009-SGR-965). References [1] C. Corcellas, E. Eljarrat, D. Barceló. 2015. First report of pyrethroid accumulation in river fish: A case study in Iberian river basins (Spain). Environ Sci Technol 75: 110-116. [2] Alonso MB, Feo ML, Corcellas C, Vidal LG, Bertozzi CP, Marigo J, Secchi ER, Bassoi M, Azevedo AF, Dorneles PR, Torres JPM, Lailson-Brito J, Malm O, Eljarrat E, Barceló D. 2012. Pyrethroids: A new threat to marine mammals? Environ Int 47: 99-106.

MO005
Target and non-target screening analysis using gas chromatography-quadrupole-time-of-flight (GC/Q-TOF) to prioritize emerging pollutants for seafood monitoring
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Human is exposed to organic contaminants with several pathways, depending on target contaminants and their usage. Seafood consumption has known as a major exposure pathway to toxic organic contaminants including persistent organic pollutants (POPs). In order to prioritize the emerging pollutants in seafood, we employed non-target screening analysis using gas chromatography/quadrupole-time-of-flight (GC/Q-TOF). Surface seawater, sediment, and seafood samples collected from Ulsan Bay, Korea, to investigate the occurrence and migration efficiency for detected organic contaminants. To make our analysis effective, we made database with 200 target chemical standards based on nation-wide monitoring programs (e.g. POPs). Non-target screening analysis was also performed to identify unknown chemicals in multi-media matrices. To remove matrix effect in each environmental sample, we developed

clean-up method based on solid phase extraction (SPE) with HLB cartridge. Using matching with NIST library and deconvolution techniques, we found approximately 4000 compounds in seawater, sediment and seafood samples. The predominantly identified compounds were siloxanes, phthalates, musk fragrances, and phosphate or chlorinated flame retardants, implying the strong candidates for seafood monitoring in Korean coastal waters. Our approach or framework for prioritization of emerging pollutants in seafood could be effectively utilized as occurrence-based prioritization in various environmental compartments.

MO006

Occurrence and assessment of Perfluorinated compounds in fisheries from Korea

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Residues of 15 perflourinated compounds (PFCs) were investigated in 58 fisheries including 31 fish from Korea. The total concentrations of PFCs in muscles of fisheries ranged from 0.04 to 32.2 ng/g wet weight. Bivalves such as mussels, oysters, and clams showed the concentrations higher than 10 ng/g wet weight. The main compounds among PFCs were perfluorooctane sulphonate (PFOS) for fish, perfluorooctanoic acid (PFOA) for crustaceans, perfluoropentanoic acid for bivalves, gastropods, and seaweeds, and perfluoroundecanoic acid for cephalopods. This suggests that bioconcentration of PFCs in marine organisms is dependent on their taxonomic features (sources of food, feeding type, and metabolism). The concentrations of PFCs in livers and intestine were greater than in muscles. The muscles of crustanceans and cephalopods had greater concentration of perfluorotridecanoic acid than intestine. We also found the difference in PFC profiles between wild fish and farmed fish. Farmed fish tended to accumulate relatively greater amounts of perfluorohexanoic acid in wild fish, while perfluorododecanoic acid was abundant in farmed fish. The calculated hazard ratio of PFCs for all fisheries muscle samples, was less than 1.0, and could be classified at safe levels for the general population. The main route of PFOS to human exposure was fish consumption, and of PFOA was crustancean consumption.

MO007

Nitrate: an Environmental Endocrine Disruptor?

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This poster presents a review of the increasing experimental evidence that inorganic nitrate acts as an environmental endocrine disruptor. The double Nobel Prize awarded Haber-Bosch process, which fixates atmospheric nitrogen to ammonia as a feedstock for agricultural nitrate-fertilizers, kick-started the Agricultural Revolution. Subsequently, environmental nitrogen emissions have increased tremendously. Even though nitrogen levels in surface waters during pre-industrial times cannot be precisely estimated it is unarguable that present day nitrate-levels (1-9 mg L^{-1} in larger European rivers) are severely above those concentrations where most aquatic life has evolved. During the past decades, deviations in wildlife reproductive hormone levels and sex ratios have been reported in organisms ranging from alligators, newts, fish and frogs to small crustaceans. Despite much research effort, no single major cause of the observed changes has been found. This poster presents a compilation of the growing experimental evidence for environmental endocrine disruption by inorganic nitrate. It furthermore describes three hypotheses regarding the mechanisms by which nitrate may cause endocrine disruption: 1) Nitrate affects homeostasis by interfering with chloride and/or iodide ion transport, 2) Nitrate is converted to nitric oxide, which affects homeostasis by altered transcription of relevant genes and/or altered activity of relevant enzymes, and 3) Nitrate acts synergistic in combination with other environmental pollutants. It is likely that different species have very different sensitivities, as they have evolved in environments of different nutrient status and have inherently different hormonal systems. Future research must therefore include different ecologically relevant organisms and provide information on threshold concentrations, developmental "sensitivity windows" as well as addressing the hypothesized mechanisms.

MO008

Fate of antibiotics in pulsed corona discharge oxidation

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Antibiotics in the environment are of concern due to the development of antibiotic resistant bacteria. Conventional wastewater treatment plants are not designed to remove micropollutants such as antibiotics. Thus, a significant amount of these compounds are released into the aquatic environment. The gas-phase pulsed corona discharge process (PCD) is an effective, environmentally friendly method for removing recalcitrant organic compounds in waste water. In PCD organic compounds are transformed through reactions with hydroxyl radicals and ozone. The objective of this work is to optimize PCD oxidation for the antibiotics sulfamethizole, doxyxycline and amoxicillin. Quantitative analyses of the antibiotics and their oxidation products will be performed and the main oxidation products will be fully characterized. A preliminary structural characterization of oxidation products was performed on the basis of molecular weight determination by a time of flight mass spectrometer, and fragmentation pattern recorded by an ion trap mass spectrometer. Final structural determination will be performed by NMR studies (^{1}H and ^{13}C NMR) on isolates obtained through semi-preparative LC fractionation of reaction mixtures. Subsequently, an exact quantification of the main products will be performed with a triple quadrupole mass spectrometer using the isolated compounds as authentic standards. The preliminary results show that amoxicillin forms nine transformation products of which five have been tentatively identified. Sulfamethizole forms many minor products of which the structures of five products have been preliminarily elucidated. Doxycycline forms two major products, both of which have been tentatively identified. The most common transformations are those where one or hydroxyl groups are added to the parent compound and ring opening of the β-lactam ring in amoxicillin.

MO009

WASTEFFECT: Life cycle management of emerging contaminants in waste with a focus on flame retardants

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Waste treatment has become a dynamic sector. In many countries, waste-handling infrastructure is changing from doing less landfilling and more incineration or recycling, in order to maximize energy and material re-capture. In addition to materials and energy, waste also contains a large variety of contaminants of emerging contaminants (CEC), and yet how landfilling, incineration, and recycling can effect environmental emissions and exposures of such CECs remains largely overlooked. Two such emerging contaminants found in increasing levels in waste include brominated flame retardants (BFRs) and antimony. The Norwegian research project WASTEFFECT focused on how these CECs and other contaminants behave in different waste streams, like landfilling, incineration and recycling. WASTEFFECT researchers sampled air and water emissions around several waste-handling facilities, and ultimately established a Norwegian waste emission inventory of BFRs and antimony in electronic-waste, car waste, plastics, glass and combustible waste. Collectively these data show the contribution of the waste sector to emissions of CEC in the environment, as well as how effectively the eliminate CECs from the environment. The main output of this research will help guide the waste management sector on which of the treatment methods provide the lowest risk in regards to emerging chemicals, and which processes could be optimized. These results are also of relevance to a better understanding of potential CEC risks to areas around and down-stream of waste handling facilities.

MO010

The occurrence of antibiotics in wastewaters, recipient waters and sediments

E.M. Kortesmäki, Abo Akademi University / Department of Natural Sciences; A. Meierjohann, Abo Akademi University / Laboratory of Organic chemistry; L. Kronberg, Abo Akademi University

In recent years, the occurrence of antibiotics in the environment has generated a major concern among the public and decision makers because of their possible impact on the aquatic ecosystem and possible development of antibiotic resistant bacteria. Antibiotics have been shown to enter the environment through target organisms' excretion mainly *via* discharges from wastewater treatment plants (WWTPs), through the use of animal manure in agricultural fields and through direct discharge from aquaculture. It has been shown that even the relatively low concentrations of antibiotics found in WWTPs and in the environment can give rise to the selection of antibiotic resistant bacteria. However, more data on the occurrence and fate of antibiotics is needed before an actual risk assessment of their impact can be done. The overall objective of the work was to determine the occurrence and fate of 17 most used antibiotics in Finland in WWTPs, in recipient waters and sediments. Twenty-four hour composite samples were collected from the influent and effluent waters in the WWTPs in three consecutive days. WWTPs were chosen to represent different profiles by their working efficiency, characteristics of their incoming wastewater and size. Additionally, surface and bottom water samples as well as sediment samples were taken from the discharge points of the WWTPs and from the Archipelago Sea (which is rich in fish farming industry). The analytical method developed for the analysis of 17 antibiotics and 3 metabolites (tetracyclines, β-lactams, macrolides, quinolones and sulfonamides) in this study combines on-line solid-phase extraction (SPE) extraction and LC-MS/MS identification and quantification (an existing pre-treatment technique of SPE with liquid chromatography mass spectrometry triple quadrupole through on-line connection). An anti-epileptic drug carbamazepine was used as a tracer for wastewater contamination. Isotopically labeled isomers of the analyzed compounds were used for quantification. Obtained results show that nine antibiotics could be detected frequently at concentrations ranging from 500 ng l^{-1} in the influent to low ng l^{-1} in the effluent waters. Clarithromycin, erythromycin and trimethoprim were found close to the discharge point (at the nearest sampling location from the discharge point) at concentrations ranging from 5 to 52 ng l^{-1}.

Preliminary results also show that some antibiotics can be detected in sediment samples taken from the Archipelago Sea.

MO011

Application of Multimedia Urban Model to estimate the environmental fate of PAHs in Tarragona County, Spain

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Concern regarding polycyclic aromatic hydrocarbons (PAHs) continues because of their ubiquity in the environment and relatively high toxicity. PAHs are emitted from incomplete or low temperature combustion processes and their environmental burdens are a function of proximity to emission sources. Numerous studies have demonstrated elevated concentrations of PAHs in urban areas. For this reason, predicting the environmental fate and transport of PAHs is an essential step in the process of assessing the impacts of these contaminants in the urban and surrounding environment. The goal of this research was to estimate the fate and dynamics of PAH in Tarragona County, Spain, that is home to the most important chemical/petrochemical industrial complex in Southern Europe. To achieve this goal we used the Multimedia Urban Model (MUM-Fate) of Diamond et al. (2001, Chemosphere 44, 1655-1667). This model is based on the Level III fugacity model of D. Mackay (Multimedia Environmental Models: The Fugacity Approach, Lewis Publishers, Boca Raton, FL, 1991) and consists of seven bulk media compartments: lower and upper air, surface water, sediment, soil, vegetation and an organic film that coats impervious surfaces. This model is characterized by considering impervious surfaces, which are a unique feature of urban environments. Here, MUM-Fate was parameterized according to conditions in Tarragona County and then run with an illustrative emission rate of 1 mol/h for six PAHs, Naphthalene, Anthracene, Phenantrene, Fluoranthene, Pyrene and Benzo(*a*)pyrene. Results from the MUM-Fate model showed that the film compartment achieves highest concentrations of PAHs studied, followed by soil, sediment and vegetation compartments in descending order. In contrast, soil and sediment were the greatest sinks for PAHs in Tarragona County, because they receive high inputs from air, water and vegetation relative to low losses. By far the greatest loss of PAH was due to advection from air, followed by photodegradative reactive losses from air. Although the general pattern was similar to that seen in Toronto, Canada, more PAH were lost via air advection and photodegradation whereas less PAH accumulated in vegetation, water, soil and sediment because of lower precipitation in Tarragona with its semi-arid climate. These results provide a first approximation of the fate of PAH in Tarragona County, an area highly impacted by the petrochemical industry.

MO012

Assessing the potential effects of reclaimed waters on aquatic organisms using a test battery with standardised and novel bioassays.

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Climatic change is causing more persistent drought periods in the Mediterranean basin as they reduce availability of water sources and increase undesirable impacts on aquatic ecosystems. The use of reclaimed waters for irrigation and aquaculture purposes is generally considered a reliable alternative for sustainable water management, particularly in those regions with high water demand vs. water availability. Recently, a large number of organic compounds, which are generally categorised as emerging contaminants, has been detected in wastewater effluent. To date, no quality criteria have been proposed for this group of compounds, so they cannot be controlled according to existing Spanish Legislation on reclaimed waters use (RD 1620/2007). Reclaimed water samples, supplied by four Spanish WWTPs that collect effluent of different origins (municipal only or municipal and industrial), which undergo distinct tertiary treatments, were used herein. Chemical characterisation was performed and 44 emerging pollutants, classified as pharmaceuticals, parabens, UV filters and endocrine disruptors, were analysed. A complete test battery was conducted to detect sublethal or chronic effects, which covered all taxonomic groups representative of the aquatic compartment. This bioassays battery included a modified standardised growth inhibition test on algae (OECD, 201), the standardised reproduction test on *Daphnia magna* (OECD, 211), a *Daphnia magna* feeding assay, an embryotoxicity and a reproduction test on *Physa acuta*, an embryo life cycle test on *O. latipes,* and a modified embryotoxicity test on *X. laevis* (ASTM, 1998). Reclaimed waters, dilutions of these waters (10%, 50%) and reclaimed waters spiked with high concentrations of a mixture of methylparaben, PFOs and fluoxetine were tested in this test battery. The results showed differences in the toxicity of samples, with good correlation between assays in some cases. The wide variety of studied endpoints enabled the complete biological characterisation of these samples, which confirmed the utility of the test battery to assess the potential effects of complex mixtures as reclaimed waters. This work was possible thanks to Spanish Ministry of Economy and Competiveness projects CTM2013-44986-R and CTM2014-52388-R.

MO013

Understanding the fate of active pharmaceutical compounds in surface waters receiving poorly or untreated sewage effluent and the development of appropriate environmental risk assessment approaches

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Active pharmaceutical compounds (API's) have been classified as emerging contaminants, and their introduction in the environment might pose risks to ecosystems. The main source of pharmaceuticals in the environment is human use and excretion or the improper of unused mediation to the sewerage system. In wastewater treatment works the lack of efficient removal of some pharmaceuticals, combined with pseudo-persistence, results in their presence in surface waters. As patient access to medicines increases in developing countries the environmental concentration of pharmaceuticals has the potential to be higher than in developed countries if the level of wastewater and drinking water treatment does not also increase. Untreated wastewater enters the environment via discharge into surface waters resulting in a downstream area characterized by high pollution, named "impact zone". Characterization of the environmental risk posed by pharmaceuticals in such an area is challenging since the formal protocol for environmental risk assessment was developed for environmental conditions largely different from the ones encountered in the impact zone; High levels of BOD, ammonia, and other potential toxicants exist in combination with low concentrations of dissolved oxygen, meaning that there is an absence of traditional species used for toxicological endpoints. Therefore, the calculation of predicted environmental concentrations (PEC) in such conditions is not relevant. The aim of the research is to obtain a comprehensive overview of the gaps regarding the fate of pharmaceuticals in aquatic environment initially starting with an extensive literature review of available data for (1) occurrence, (2) degradation rates, (3) partitioning to dissolved organic matter, colloids and suspended solid matter, and (4) relevant endpoints. This will inform experimental research aimed at gaining data about partitioning and degradation of selected pharmaceuticals in the impact zone at varying dilutions, and determination of proper endpoints. The information obtained will be used to develop an environmental risk assessment approach for impact zones, as only with more accurate exposure concentrations and impact data can the risks to the aquatic environment of APIs be quantified.

MO014

The Chemical Investigations Programme Phase 2: an assessment of the presence of pharmaceuticals in wastewater treatment works influent and effluent

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There is increasing interest in the presence of Active Pharmaceutical Ingredients (APIs) in the aquatic environment due to their potential environmental impact. The Water Framework Directive (WFD) "Watch List" currently includes three APIs (E2, EE2 and diclofenac) with the potential for inclusion of further such compounds in future revisions of the Directive. Wastewater treatment works (WwTWs) effluent is the primary source of APIs into the environment. The Chemical Investigations Programme (CIP) Phase 1 took place between 2007 and 2013 and examined the presence of a range of trace substances in WwTW influent and effluent, including selected APIs, across mainland UK. The results of this large-scale study indicated the need for further study to examine issues at a more localised scale and consider solutions. The CIP Phase 2 started in 2015 and includes monitoring at 600 WwTWs over a period of 5 years across England and Wales. The study encompasses monitoring of 23 key APIs, such as propranolol, carbamazepine, ibuprofen, tamoxifen and four antibiotics. This poster will present an outline analysis from the first "tranche" of results from the CIP2 study focusing on APIs in influent and effluent. Comparisons with Predicted No Effect Concentrations (PNECs) which have been developed for each API will also be presented, taking into account the dilution of each WwTWs effluent, as well an assessment of removal during the treatment process at different sites.

MO015

Presence of virulence genes in Enterococcus spp. isolated from South African environmental water systems

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Enterococcus spp. from environmental sources harbouring virulence factors may not always express functional gene products. However, their presence may be

indicative of potential pathogenicity and could also contribute to the spread of these genes in the environment. The aim of this study was to determine the relationship between the presence of selected virulence genetic determinants and the expression of these factors from environmental water from the North West province of South Africa. A total of 93 enterococcus isolates, consisting of six species, were screened for the presence of 5 virulence determinants (*asa1, cylA, esp, gelE* and *hyl*) using PCR (polymerase chain reaction). Furthermore, the expression of *cylA, hyl* and *gelE* were determined by phenotypic assessments illustrating β-haemolysin, hyaluronidase and gelatianse activity. Seventy percent of the screened isolates harboured at least 1 virulence gene whilst 15% harboured 2 or more. The most frequently detected virulence genes were *cylA* and *gelE*. However, *asa1* and *hylA* were also detected. Enterococcal surface protein (*esp*) was the only virulence determinant absent in all screened *Enterococcus* spp. The presence of virulence genes was correlated with their extracellular enzyme production. Only 40%, 22% and 22% of the *cylA, gelE* and *hyl* gene carrying isolates produced β-haemolysin, gelatinase and hyaluronidase, respectively. The results of this study show that a large percentage of these environmental enterococci (including E. *faecalis, E. faecium, E. mundtii, E. casseliflavus, E. gallinarum* and *E. hirae*) possess virulence factors and that these could be expressed *in vitro*. This could have implications for individuals using this water directly for recreational and other purposes. Therefore measures to minimize their presence in such water sources are vital. **Keywords:** *Enterococcus* spp.; environmental water systems; virulence genes; extracellular enzyme production

MO016

OCCURRENCE OF 16 ILLICIT DRUGS IN CEYHAN RIVER, TURKEY

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Illicit drug concentrations in surface waters which under the influence of waste water treatment plants can be an indirect tool to estimate the community level of consumption of illicit drugs. These drugs enter the surface waters unaltered or slightly transformed via waste water effluents by human excretion after illegal consumption, by intentional extermination from undercover drug laboratories or by accidental. The illicit drugs and their metabolites are very recalcitrant to elimination at conventional waste water treatment plants. In the current study, which is one of the first studies in Turkey, presence and seasonal variability of 16 illicit drugs and metabolites in Ceyhan River, which places in Southern Turkey was investigated. Water samples were collected from 9 stations at seasonal intervals for one year to characterize the seasonal variability of drugs. One of these stations was near the Osmaniye waste water treatment plant. As a part of this study, a sensitive and selective liquid chromatography-tandem mass spectrometry (LC-MS/MS) screening method targeting 16 illicit drugs has been developed and employed to investigate the occurrence of these drugs in water samples. Solid phase extraction techniques were used for all samples. 16 different illicit drugs and metabolites (6-MAM, cocaine, codeine, fentanyl, hydrocodone, hydromorphone, JWH-018, JWH-073, ketamine, MDA, MDMA, amphetamine, methamphetamine, methylecgonine, morphine, tramadol) were examined with LC-MS/MS. Fourteen of these drugs couldn't be found in the river water samples. Cocaine and tramadol were detected in 77.8% of the river water samples. The concentrations found in the water samples in the low ng/L range. Median cocaine concentration was 0.404 ng/L, and tramadol concentration was 0.420 ng/L. Highest illicit drug concentrations were detected in the station close to the wastewater treatment plant and summer season. Environmental concentrations of these illicit drugs are low, but they may be toxic to the aquatic organisms. Risks to the environment and human health are under consideration and further studies about the occurrence and toxicology should be done.

MO017

Mixture toxicity effect of bisphenol AF and sulfamethoxazole on thyroid endocrine system in zebrafish

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Bisphenol AF (BPAF) is widely used in the production of polycarbonate copolymers as an alternative of bisphenol A. Given the complex nature of mixtures of environmental pollutants in aquatic systems, the present study aimed to evaluate potential for thyroid endocrine disruption in fish co-exposed to BPAF and sulfamethoxazole (SMX). Adult male zebrafish were exposed to control, BPAF (50 μg/L), SMX (50 μg/L), and a binary mixture of BPAF and SMX for 21 days, and the effects on growth and tissue somatic index were examined. Concentrations of thyroxin (T4) and expression of mRNAs for 10 functionally relevant genes of the hypothalamic-pituitary-thyroid axis were also measured. Microarray analysis was performed to identify altered genetic targets by pooling total RNAs from three individual fish in each treatment group. Significant increase of thyroid somatic index was observed in fish exposed to a mixture of BPAF and SMX. Expressions of *trh*, *tshr*, and *tshβ* genes in thyroid were significantly increased when fish were co-exposed to BPAF and SMX. With a combined exposure to BPAF and SMX, the extent of increase in gene expressions and T4 hormone production were more pronounced than those of BPAF only exposure group. Moderate correlation between microarray and gene expression values was observed. The present study indicates that combined exposure to SMX could significantly increase an endocrine disrupting effect of BPAF.
Acknowledgement – This study was supported by National Research Foundation of Korea (Project no. NRF-2013R1A1A1061684).

MO018

Toxic and genotoxic potency of rainwater samples from Greece on bacteria and human cells

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Rainwater is considered as the main route of pollution from the atmosphere to the aquatic ecosystems. Despite the significant effect of rainwater quality on the different levels of trophic chain, a relatively small number of studies has been conducted regarding the impact of rainwater to living organisms and microorganisms. The main focus of the present work is to provide novel knowledge in relation to the potential adverse effects of rainwater on aquatic ecosystem and human health. In this context, the toxic and genotoxic effects of various rain water samples collected from three urban cities of Western Greece were estimated. Acute toxicity of the rainwater samples was evaluated by monitoring the inhibition of the luminescence emitted by the marine bacterium *Vibrio fischeri,* using a Microtox system (Azur Environmental). The cytotoxic and genotoxic potential activity of rainwater samples was assessed employing the cytokinesis block micronucleus (CBMN) assay. The physicochemical composition of the samples was also determined. The pH values of rain samples ranged between 5.8 and 7.2, indicating a reduction of the pH index during the summer months. In contrast, conductivity increased, in the same sampling periods. Chemical analysis revealed that SO_4^{2-} followed by Cl^- were the dominant anions in the majority of the samples. The increased concentration of chloride ions in the rainwater samples from cities situated in coastal zones is probably correlated with the transport of seawater aerosol. Concerning the % inhibition of luminescence of the bacterium *Vibrio fischeri,* the highest percent of inhibition was observed to the samples collected during the summer months. The results of the CBMN assay showed an induction of genotoxicity in the rainwater samples on the month of December in all urban areas, as well as a cytotoxic activity in the majority of the samples with a most pronounced increase during the summer months. Correlation between obtained toxicity data and chemical composition of the samples proved significant contribution of ions that originate from anthropogenic sources (nitrogen ion species) in the observed toxic effects.

MO019

Occurrence of seven bisphenol analogues in paper products from Korea: Implications for regulation and human exposure

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Bisphenol A (BPA) has been produced for the past decades with wide variety of commercial products, such as polycarbonate plastics and epoxy resins. Humans are mainly exposed to BPA through diet and dermal absorption from the use of paper products is also considered as a major exposure route to BPA for humans. Recently, BPA has been used in paper products including thermal receipt paper, currency bills, and business cards. As the concerns for toxic effects of BPA grows, BPA is gradually being replaced to related compounds such as bisphenol S (BPS) and bisphenol F (BPF). However, limited information is available concerning the occurrence of bisphenol analogues (BPs) in the paper products. In this study, the concentrations of seven BPs (BPA, BPS, BPF, bisphenol AF (BPAF), bisphenol AP (BPAP), bisphenol Z (BPZ), bisphenol P (BPP)) were measured in six types of paper products (n = 153), including thermal receipts, paper currencies, business cards, airplane boarding passes, cafe coupons, and newspapers collected from Korea. In all paper products, BPA showed the highest detection rate (88%), followed by BPZ (47%) and BPS (16%). Among paper products, thermal receipt contained the highest levels (mean: 2.19 mg/g) of BPA, compared with other paper products (mean: 1.5 μg/g). In particular, seventeen thermal receipts (14% of total) showed the higher levels of BPS (mean: 1.04 mg/g) than BPA (mean: 0.017 mg/g), implying the evidences that BPA is replacing into BPS. These 17 receipts have 'BPA-free' labeled in common on the back of receipts. Our findings indicate that BPA is still used as a predominant analogue in thermal receipt papers, but BPA is replaced into BPS. The daily intake (EDI) of BPA associated with dermal absorption of paper products was estimated to be 2.91 ng/day for general population and 8905 ng/day for occupationally exposed populations in Korea. Among paper products, thermal receipt papers contributed to most (>70%) of the BPA exposures for dermal consumption. Considering high occupational exposure groups, survey and risk assessment for the exposure of BPs in thermal receipt would be necessary.

MO020

QuECHERS as perspective extraction technique for isolation of steroid

estrogens from sediments in environmentally relevant concentrations

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The presence of endocrine disrupting compounds including steroid estrogens in water environment is worldwide problem. These compounds are released from different sources such as wastewaters from household through ineffective wastewater treatment plant also as result of runoff from agricultural areas after animal manure applications. Steroid estrogens cause a negative effect on growth, development and reproduction of aquatic organisms at very low concentrations. Due to higher octanol-water partition coefficient they tend to sorption and accumulation to solid matrices such as sediments. For a study of estrogens behavior in water-sediment system the effective extraction technique is needed. For this reason the four extraction technique: Soxhlet warm extraction (SWE), Accelerated solvent extraction (ASE), Microwave assisted extraction (MAE) and Quick Easy Cheap Effective Rugged Safe (QuCHERS) were compared using different extraction conditions. Estron (E1), 17α – estradiol (αE2), 17β- estradiol (βE2), estriol (E3), 17α-ethinilestradiol (EE2) and 17α-ethinilestradiol 2,4,16,16-d4 (EE2d4) were extracted from sediment samples at very low initial spike concentrations (1ng/g). Wider range of extraction recoveries (40-120 %) was considered as acceptable in those cases. Simple, fast and inexpensive original QuECHERS with PSA clean up provided the best extraction recoveries for all tested estrogens (53-84 %). Also MAE achieved the satisfactory extraction efficiency using DCM:acetone 3:1 as extraction solvent (50-70 %) but only for less polar estrogens. For estriol the more polar extraction solvents (acetone or methanol) must be used. More time consuming extraction technique ASE provided lower extraction recovery or worse repeatability in almost all solvent-temperature combinations in comparison with previous extractions. The worst results were obtained by most time consuming technique SWE that is totally unsuitable for this purpose. This comparison also showed that the correct choice of internal standard for calculation of final estrogens concentrations is very important due to great complexity of sediment samples. Deuterated 17β-estradiol was firstly used in SWE and ASE as internal standard for all tested estrogens but these compounds cause an overestimation of E3. For this reason deuterated 16α-hydroxy-17β-estradiol was selected as preferable internal standard for E3. *Acknowledgment: Reserch was funded by GACR GA13-20357S*

MO021

Transformation products of quetiapine formed by UV treatment and biodegradation

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Quetiapine (QUT) arouse much attention in recent years for the treatment of schizophrenia and bipolar disorders. As QUT is consumed in high amounts and is not efficiently removed by sewage treatment plants (STPs), it has been frequently detected in secondary effluent. UV treatment could be a possible advanced treatment option to eliminate QUT. Moreover, the release of QUT to surface water is very likely, and therefore, the fate regarding biodegradability of QUT needs to be assessed. UV treatment of QUT in ultrapure water at different concentrations (86.9, 17.4 and 4.3 mg/L) was conducted. The primary elimination and the mineralization was monitored for 128 min by means of HPLC-UV and NPOC, respectively. QUT and the photolytic mixture after 128 min of UV treatment were submitted to biodegradation tests using different bacterial density and test compound concentration, namely the Closed Bottle Test (CBT; OECD 301D) and the Manometric Respiratory Test (MRT; OECD 301F). Structures of photo- and biotransformation products (PTPs and BTPs, respectively) were elucidated by high-resolution mass spectrometry. At high initial concentration of 86.9 mg/L, QUT was not completely eliminated after 128 min of UV treatment. Primary elimination followed zero order kinetics ($t_{1/2}$ = 100 min). NPOC recovery was 97% indicating the formation of PTPs. The elimination half-life increased at initial concentration of 17.4 and 4.3 mg/L fitting a first order model ($t_{1/2}$ = 24 and 5 min, respectively), while NPOC recovery was 95 and 30%, respectively. In the CBT and the MRT, QUT was not readily biodegradable according to OECD test criteria (16.6 ± 1.5% and -1.9 ± 3.2%, respectively), while QUT was primary eliminated to some degree (20.2 ± 3.9% and 88.8 ± 10.9%, respectively) indicating the formation of BTPs. The extent of biodegradation regarding biochemical oxygen demand (BOD) of the photolytic mixture was not increasing compared to QUT. Several PTPs and BTPs have been identified and structurally elucidated. PTP 400 (sulfoxide derivative) and BTP 398 (carboxylic acid derivative) are also formed to a high extent as human metabolites. UV treatment of secondary effluent contaminated with QUT could result in formation of PTPs. These PTPs cannot be generally seen as better biodegradable. Therefore, UV treatment should be considered critically as a possible treatment option in this respect. BTPs of QUT should be further characterized regarding their potential toxic effects in the environment.

MO022

Bisphenol A alternatives cause reproductive endocrine disruption in zebrafish (Danio rerio)

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Although the toxic effects of waterborne bisphenol A (BPA) exposure were frequently studied in aquatic organisms, limited information is available on the toxicity of BPA alternatives, such as bisphenol analogues or Tritan^{TM} copolyesters. In the present study, reproductive endocrine disruption of bisphenol AF (BPAF), bisphenol S (BPS), and one of the main component of Tritan^{TM} copolyesters (1,4-cyclohexanedimethanol (CHDM)) in zebrafish were investigated. Adult zebrafish pairs were exposed to environmentally relevant concentrations of BPAF (0.5~50 μg/L), BPS (0.5~50 μg/L), and CHDM (0.1~10 μg/L) for 21 d, and the effects on reproduction, sex steroid hormones, and transcription of the genes belonging to the hypothalamic-pituitary-gonad axis were examined. The adverse effects on performances of F1 generation were further examined. The average number of eggs spawned was significantly less upon the exposure to ≥0.5 μg/L BPAF and BPS, while no significant changes were observed in fish exposed to CHDM. Exposure to two bisphenol analogues resulted in estrogenicity in male fish, showing significant increase in plasma 17β-estradiol (E2) concentration. The increase of E2 is well supported by significant up-regulation of aromatase (cyp19) gene, which catalyzes the final step in conversion of androgen to estrogen. Parental exposure to BPAF and BPS resulted in delayed hatching and increased malformation rates. Given the importance of endocrine function, further investigations on underlying mechanisms of sex-dependent responses in zebrafish are needed. Acknowledgement – This study was supported by National Research Foundation of Korea (Project no. NRF-2015R1D1A1A01056628).

MO023

Characterization of samples by endocrine activity profiles using the planar yeast estrogen screen (pYES)

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Recently, a protocol for the direct combination of thin layer chromatography and the yeast estrogen screen (pYES) was developed as a screening tool for effect directed analysis (EDA). The advantages of this approach lie in its rapidness compared to an EDA-approach with e.g. HPLC and a subsequent analysis of fractions for biological activity and in its robustness. The method allows for the analysis of effects in demanding matrices. The pYES supports compound identification by the possibility to quickly falsify alternative compounds and by the direct accessibility of the compounds on the TLC-plate to a subsequent analysis by mass spectrometry. Qualitative and quantitative results about specific effects in mixtures can be obtained within one working day. Activity profiles of samples can be generated easily which allow a comparative assessment of alternative processes for e.g. wastewater treatment without a detailed chemical analysis. Furthermore, sources of environmental contamination can be identified and characterized based on biological effects rather than compounds, i.e. a source-identification is independent from the knowledge about defined chemical compounds. Due to the high sensitivity of the method it is possible to detect less than 30 pg/l 17a-ethinylestradiol and 17b-estradiol after a 1000-fold concentration. The pYES might thus serve as a screening tool for the characterization of these compounds in surface waters according to the watch list of the EU water framework directive. Taken together, the pYES-approach seems to have a high potential to be used as a fast and robust screening tool for various applications in effect directed analysis. The approach is complementary to the more common combination of HPLC with a subsequent bioassay.

MO024

Evaluation of the acute toxicity and genotoxicity of pyrolytic tire chars

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The disposal of waste tires constitutes a major problem with environmental and financial impact worldwide. The last decades, pyrolysishas received considerable attention for the management of waste tire disposal resulting simultaneously in the recovery of valuable productssuch as oil and gas fractions, as well as pyrolytic char. Pyrolytic char from tire-rubber (PyrolyticTyre Char, PTC) is a carbon material similar to carbon black (CB), extensively used as adsorbent or support. As adsorbent, PTC has exhibited promising results for the removal of organic and inorganic pollutants from aqueous media. In recognition of its potential application in aquatic remediation, in the present study toxicity, genetoxicity and cytotoxicity potential of PTC (untreated and acid (HNO_3-treated) was investigated

for the first time. The acute toxicity of PTCs was evaluated using the marine bacteria *Vibrio fischeri*, which emit bioluminescence as a byproduct of cell respiration and normal metabolic process, by a Microtox Model 500 Analyzer (AzurEnviromental). In each experiment, the % inhibition of the luminescence and the EC_{50} values (mean sample concentration that causes a 50% reduction in bacteria bioluminescence) were recorded after 5, 15 and 30 min of incubation. The genotoxic and cytotoxic activity of the untreated and acid-treated PTC was studied applying the cytokinesis block micronucleus (CBMN) assay in human lymphocytes *in vitro*. Both the untreated and acid-treated PTC were studied at three different doses i.e. 2.5, 5 and 10 mg L^{-1}.The EC_{50} values for the untreated and acid-treated PTC after 30 min of exposure were determined as 486 and 163 mg L^{-1}, respectively. Both the untreated and acid-treated PTC were found to be genotoxic at all the tested concentrations in the CBMN assay. In the case of acid-treated PTC a slight decrease in MN frequency was observed. Regarding the cytotoxicity, both PTCs induced cytotoxic activity in all the tested concentrations.

MO025

A comparison of micropollutant removal in different biological wastewater treatment plants

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Concerns over the effects of chemicals in the aqueous environment has seen the introduction of more stringent water quality regulations and tertiary treatment using advanced oxidation processes such as ozonation. These interventions make existing wastewater treatment more energy-intensive; aeration in activated sludge alone accounts for up to 1.5% of UK electricity use. The transition to carbon-neutral wastewater treatment will require the adoption of low-energy biological treatment systems that operate with little or no active aeration, such as those extensively used in Latin America (upflow anaerobic sludge blanket reactors; UASBs and waste stabilisation ponds; WSPs). Little is known about the true biological limits of micropollutant removal especially in low-energy systems. In this study we wanted to investigate a range of structurally diverse micropollutants with varying degrees of halogenation towards that goal. We started by validating analytical methods for the quantification of triclosan, PAHs and PBDEs using SPE-GC-MS and SPE-LC-MS and using them to assess triclosan and PAH removal in two UK WWTPs. Recoveries of the compounds in wastewater was between 70 – 130% with a relative standard deviation of ≤ 20. Method detection limit of triclosan in effluents was 5 ng/L, while low molecular weight PAHs was 0.4 – 1.2 ng/L and high molecular weight PAHs was between 4 and 12 ng/L. The concentration of triclosan in effluents from both WWTPs was above the Predicted No-Effect Concentration (PNEC) of triclosan in freshwater (i.e. 100 ng/L). PAHs concentration in the effluent exceeded their EQS standards and about 90% of total PAH concentration partitioned in the suspended particulate matter. We are currently determining first order biodegradation rates for the chemicals under aerobic and anaerobic conditions, and will assess their fate and removal in real UASBs and WSPs from Brazil compared to conventional systems.

MO026

Extended application of a human pharmaceuticals emission model to the assessment of metabolites.

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Accurate prediction of emission is critical to assessing and managing exposure to pharmaceuticals in freshwater, particularly because extensive monitoring data are often lacking. In our previous work (1), an emission prediction model was presented with the uncertainties associated with its prediction of human pharmaceuticals discharged into the typical Korean river environment. The model covers the stages of pharmaceutical life cycle posterior to domestic production and import, including distribution, consumption, disposal, and waste treatment. In the present work, the emission model was combined with SimpleBox and SimpleTreat into a single spreadsheet-type model for calculating the predicted environmental concentrations of the human pharmaceuticals and their metabolites. For metabolites, the stages of life cycle were appropriately adjusted from after intake of the parent pharmaceutical through to the discharge. This model was then used to determine the whole hazard quotients by taking into account not only human pharmaceuticals but also their metabolites in surface water of Korea. From all the human pharmaceuticals sold above 1,000 kg in 2011 in Korea, 6 pharmaceuticals (acetaminophen, allopurinol, cefepime, furosemide, ofloxacin, and theophylline) were selected of which metabolite information (species, formation rate, and excretion rate) is available from public database. As predicted no effect concentration data of the selected pharmaceuticals and their metabolites are scarce, ECOSAR was used for toxicity estimation. The metabolites of acetaminophen and theophylline were found to have the hazard quotients that are of comparable magnitude with those of their parent pharmaceuticals. As the finding strongly suggests a need to assess the environmental risk posed by metabolites, the model developed in the present work can be used as a quantitative tool to efficiently screen the human pharmaceuticals concerning the potential risk from both the parents and their metabolites. Reference (1) Eun Jeong Han, Hee Seok Kim, Dong Soo Lee; Environ. Health. Prev. Med., 19(1), 46-55, 2014

MO027

Exploring the use of synthetic musk fragrances as tracers of wastewater discharges in surface waters

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Traces of almost all the substances consumed by humans or released by anthropic and industrial activities end up in the environment and if measured in the different environmental compartments can give some information on their sources. The need for determining the contribution of the different sources of contamination in a polluted and urbanized area in Northern Italy drove us to search for tracers of specific sources in order to find a quantitative correlation with specific human activities. Anthropogenic compounds previously suggested as potential wastewater indicators include artificial sweeteners, persistent drugs, and personal care products. Since each of these tracers has different uses, human metabolism and transformation pathways in natural systems or in treatment plants, the simultaneous analysis of different tracers with their metabolites and transformation products under different hydrological and precipitation regimes can be a powerful mean to assess the relative contribution of different sources and processes. In the framework of a wider project aimed to compare different urban tracers, we developed and tested an Isotope Dilution GC-MS method for the analysis of synthetic musk fragrances in waste and surface waters. Synthetic musks are widely used as fragrances additives in many consumer products, they are widely present in the environment and they tend to accumulate in sediment, sludge and biota. The method, validated taking carefully into account the problem of blank contamination, allows to determine 9 compounds, including nitro-, polycyclic-, macrocyclic musks and a transformation product (galaxolide lactone), which can be a specific tracer of WWTP discharge. The occurrence of this class of substances has been evaluated in the river Po basin, which is an area with a very high pressure from population, industry and intensive agriculture, through specific campaigns that will be designed to evaluate their suitability as markers of anthropic pollution. Treated wastewater from wastewater treatment plants of Milano and Monza, surface waters (River Lambro and Lambro Meridionale) and irrigation waters has been included in the analytical campaign to assess the overall mass balance of the selected tracers.

MO028

National Environmental Specimen Bank of Norway

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The modern society are probably using chemicals today that will be regarded as pollutants in the future. The samples stored in Environmental Specimen Bank (ESB) Norway can be used to identify the presence and/or biological effects of such substances in the environment alongside development of new analytical methods for detection. ESB Norway contains frozen samples of animals, plants, air and mud from across Norway and the Arctic. Sample collection is coordinated with routine monitoring programmes, ensuring a consistent sampling strategy and possibility to correlate currently monitored parameters to future results. During 2016, the bank will open for applications for sample acquisition for the first time, marking the starting point of new knowledge as the interest rate from stored samples.

MO029

Transfer of POPs from amendments to agricultural soils

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Recycling organic waste as soil amendments is beneficial for organic matter, soil organisms and soil structure. The ECOSOM project study the effects on soil related to application of different organic wastes or amendments. Studies on persistent organic contaminants (POPs) are important since there is an interesting and complex interplay between organic contaminants and soil organic matter. In this project soil at two French sites have been amended with composted municipal solid waste, composted biowaste, composted sewage sludge and farmyard manure and soil at two Swedish sites were amended with sewage sludge or compost. Organic waste products doses input on the different field sites in this study did exceed usual doses applied in France and Sweden. Analysis of polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/F), polybrominated dibenzo-p-dioxins and dibenzofurans (PBDD/F), polychlorinated biphenyls (PCB) and polybrominated diphenyl ethers (PBDE) were performed on soils and amendments. Soils and amendments were Soxhlet-Dean-Stark extracted then purified and fractionated on two sequential gravity fed liquid chromatography columns, a multi-layer silica gel column and a Florisil® column. The samples were analyzed using a Waters AutoSpec ULTIMA NT 2000D high resolution mass spectrometer. Amendments used in the field trials all contained the targeted POPs, but the contents are mainly low and vary greatly between the different types of amendments and the POPs concerned. Sewage sludge had the highest combined toxic potential (of PCDD/F, PBDD/F and WHO- PCBs) at 20 ng WHO-TEQ kg^{-1} amendment. The concentrations of the targeted POPs increased in most amended soils at the four field sites. However, the observed increase were inferior to the flows of POPs provided by the amendments. This may be explained by the degradation of the compounds, their interaction with soil organic matter which render them unrecoverable or their transport into deeper soil layers. However, the results from one of the field sites in Sweden differ from the other field sites in the study. It was the only site were the combined toxic concentration increased. The increase were 12 times, 36±1.4 ng WHO-TEQ kg^{-1} soil compared to 3.1±0.5 ng WHO-TEQ kg^{-1} soil in the control soil. All the targeted compounds, PCDD/F, PBDD/F, PCB and PBDE have increased in concentration with 14, 2, 85 and 68 times since the establishment in 1956 compared to the concentrations in the control soil today.

MO030

Determination of Rodenticides in Fish Samples of the German Environmental Specimen Bank

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For the determination of eight rodenticides in fish muscle and liver an appropriate method was adap¬ted. Initial analyses revealed that higher concentrations were detected in fish liver as compared to fish filet. Thus the method was further optimized for liver samples and validated. The final protocol applied high-resolution mass spectrometric detection and yielded a limit of quantification (LOQ) of 0.06 µg/kg wet weight for most compounds (validated by repeated measurements). Applying the final method to bream liver samples from the German Environmental Specimen Bank (ESB), Bromadiolone, Flocou¬mafen, Brodifacoum, and Difethialone were found at levels > LOQ. In a further step, bream liver samples from the ESB archive sampled at 17 sites across Germany were analysed retrospectively. In this spatial comparison for the year 2011, highest levels were found at the site Saar / Rehlingen: 0.8 µg/kg Bromadiolone, 4.6 µg/kg Brodifacoum, 4.0 µg/kg Difethialone. These three compounds were the only identified rodenticides. Brodifacoum was the most frequently detected rodenticide at all test sites (found at 10 of 17 sites). The other rodenticides occurred rarer (Bromadiolone at 3, Difethialone at 7 locations). Difethialone reached a concentration of 4.0 µg/kg in bream liver from the site Saar / Rehlingen. Based on the results of the rodenticide spatial screening, samples from Saar / Rehlingen and Elbe / Prossen were chosen for a temporal comparison. From both sites, ten bream liver samples from the ESB archive were retrospectively analysed (years 1992 to 2013). Examining the results, no clear trend can be observed, but year-to-year changes in rodenticide loads. Again, Brodifacoum was the most frequently detected rodenticide (detected in almost every investigated year at Elbe / Prossen), and the most abundant one (levels of up to 4.6 µg/kg at the site Saar / Rehlingen 2011). Bromadiolone was found only in some years (up to 1.8 µg/kg, Elbe / Prossen 2003). Difethialone was detected only in some years (up to 4.0 µg/kg bream liver, Saar / Rehlingen 2011).

MO031

The impact of natural and anthropogenic Dissolved Organic Carbon and pH on the toxicity of triclosan to Gammarus pulex

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Routine ecotoxicology testing rarely accounts for the effect of natural water chemistry on chemicals bioavailability. Therefore, this study identifies whether key omissions in relation to Dissolved Organic Carbon (DOC) and pH have an impact on calculated effect concentration (EC) values. Laboratory ecotoxicology tests were undertaken for the widely used antimicrobial compound triclosan, using wild Gammarus pulex. These tests were undertaken in synthetic fresh water, humic acid solutions and wastewater treatment works effluent at mean pH values of 7.3 and 8.4. G. pulex immobilisation and triclosan concentrations were measured and used to calculate 24 and 48 hour EC values. The results showed that toxicity tests undertaken at a pH above triclosan's pKa and in the presents of humic acid and effluent, containing 11 and 16 mgl^{-1} mean DOC concentrations respectively, resulted in decreased triclosan toxicity. This was most likely a result of varying triclosan speciation and complexation, due to its pKa and high hydrophobicity controlling its bioavailability. The mean 48 hour EC50 values calculated under different conditions varied between 0.75 and 1.93 mgl^{-1}. These results demonstrated that both pH and DOC can have a statistically significant effect on triclosan's toxicity. This suggests that standard ecotoxicology tests can cause inaccurate estimations of triclosan's bioavailability and subsequent toxicity in natural aquatic environments. This could result in tests overestimating toxicity, causing overly stringent Environmental Quality Standards. These results highlight the need for further consideration regarding the role that water chemistry has on the toxicity of organic contaminants and how ambient environmental conditions are incorporated into the standard setting process in the future.

MO032

Sun light enhances toxicity of methyl paraben and 1,2-hexanediol to Daphnia magna and Danio rerio embryo

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Methyl paraben (MP) has been widely used as antimicrobial preservatives in cosmetics, pharmaceuticals and food products, and 1,2-hexanediol (1,2-H) has been applied for cosmetics as an alternatives of paraben. However, very limited information has been reported on the phototoxicity of these chemicals among aquatic organisms. In the present study, acute toxicities of MP and 1,2-H were evaluated using Daphnia magna (48 h exposure) and Danio rerio (96 h exposure) embryos. We also investigated whether phototoxicity of MP and 1,2-H would be affected by environmental level of ultraviolet lights, i.e., exposure to 4 h/d sunlight. Changes in expression of genes related to oxidative stress were determined in D. magna juveniles after being exposed to sublethal levels of the chemicals and environmental level of ultraviolet lights. Greater toxicities were observed with MP and under environmental level of ultraviolet lights in both species. In D. rerio embryos, exposure to MP resulted in lesser rates of hatching and increased malformation rates. Expressions of hemoglobin and α-esterase genes in D. magna were increased by co-exposure to MP and to ultraviolet lights. Our experiments demonstrated that exposure to MP led to oxidative damage in aquatic organisms and it was aggravated by natural ultraviolet lights. Further assessment for sublethal effects and specific mechanisms of MP under environmentally relevant conditions should be followed. Keyword: Phototoxicity, methyl paraben, 1,2-hexanediol, ultraviolet light, oxidative stress

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MO033

Evaluation of decamethylcyclopentasiloxane (D5) accumulation behavior in Herring gull eggs from the Oslofjord urban environment

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Since its initial detection within the Oslofjord in 2005, decamethylcyclopentasiloxane (D5) has been the focus of monitoring and regulatory activities over the past several years. Through the combination of high production/use and hydrophobicity/lipophilicity, accumulation of D5 in aquatic environments (e.g., sediment, biota) have not only been observed in the Oslofjord, but many other aquatic ecosystems impacted by human activities and waste. Aquatic species are considered to be at greatest risk to cVMS exposure due to exposure through sediment compartments and lower elimination capabilities. Monitoring and research focus has mostly been placed on aquatic environments as cVMS accumulation potential in air respiring organisms is considered low due to high elimination through metabolic degradation and respiration processes. However, recent findings under the "Environmental Contaminants in an Urban Fjord" program of the Norwegian Environmental Protection Agency showed elevated levels of cVMS within Herring gull eggs collected from the Oslofjord region. Concentrations of D5 detected in Herring gull eggs collected in 2013 (51.6 ± 71.8 ng/g wet weight (ww)) and 2014 (61.4 ± 51.7 ng/g ww) were comparable with maximum concentrations reaching 265 ng/g ww. Similar levels were observed for PCB 153 in 2013 (130 ± 96.2) and 2014 (75.8 ± 75.1 ng/g ww), indicating substantial uptake of D5 from the surrounding environment. High variation in ^{15}N stable isotope signatures indicates a variable eating pattern among mother birds. In addition, both ^{13}C and ^{15}N stable isotope signatures were not reflective of the local aquatic food web, indicating feeding of the mother birds has occurred elsewhere. Low ^{13}C and ^{15}N stable isotope signatures in Herring gull eggs may indicate greater feeding from terrestrial based carbon sources for energy. As opportunistic feeders, Herring gulls are known to scavenge on human waste, which are highly contaminated with volatile siloxanes and may represent an important source of D5 exposure to seabirds. Although air breathing organisms are considered to be of low risk for the bioaccumulation of D5, results presented here indicate D5 acumulation occurs within herring gull eggs. This is may indicate a potential risk to un-hatched birds and should be further monitored.

MO034

Emerging pollutants in reclaimed water. Is there some concern about crop irrigation?

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Reclaimed water is a strategic effective way to help solve the problem of water scarcity and droughts in the EU. In 2009 in Spain, the current level of water reuse was estimated to be about 500 Mm^3 (Spanish Ministry of the Environment). It is predicted that wastewater reuse in Europe will be 3,222 Mm^3/year by 2025, with Spain in first position (over 1,200 Mm^3/year). Agricultural irrigation is the main application in many EU countries and accounts for around 33% of total water use (EEA, 2012), although this figure comes close to 60% in certain regions of Spain. In this work, reclaimed water samples supplied by a Spanish WWTP were used to analyse the presence of 44 emerging pollutants (pharmaceuticals, parabens and endocrine disruptors). Different microcosms and semi-field studies were performed with three crops (lettuce, corn and radish) irrigated with spring water, reclaimed water and fortified (atenolol, carbamazepine and triclosan,) reclaimed water (at 10 and 100 higher levels than the mean observed in reclaimed waters). The aim was to assess possible adverse effects of emerging pollutants on soil-plant systems caused by reclaimed water. The experimental results evidenced no variations in soil functionality, pigments content in leaves or accumulation of emerging pollutants in different plant parts compared to those irrigated with spring water. This work was possible thanks to Spanish Government Grants CTM2013-44986-R and CTM2014-52388-R.

MO035

Use of Watson Database for prioritization of pharmaceuticals in Dutch effluents of WWTP's: a case study

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Dutch surface waters are polluted by a wide range of micro pollutants originating from several diffuse and point sources. Especially emerging compounds (EC's) may pose an ecological and human health risk, even at low concentrations. Nonetheless, relevant EC's are often not regulated with threshold values in surface waters up till now. Furthermore, basic information on ecotoxicity, concentrations and loads are often lacking. This hampers the realisation of reduction plans for emissions and therefore mitigation of risks. The effluents of Waste Water Treatment Plants (WWTP's) are identified as an import point source of EC's such as organic micro pollutants, pharmaceuticals, personal care products and illicit drugs. The Dutch Ministry of Infrastructure and the Environment therefore assembled a database containing approximately 174.000 chemical measurements (1990-2015) of 1.010 compounds in influents and effluents of 150 WWTP's. The Watson database is updated once every 3 years with measurements from regular monitoring and pilot investigations. Compounds include predominantly organic micro pollutants, but metals as well. This so called Watson database is readily available for water managers and offers estimations of concentrations, loads and removal rates of WWTP's as input for water models and thus decision making. Human pharmaceuticals are an important group of EC's and are currently investigated in The Netherlands as part of a chain approach to reduce emissions from WWTP's. The Watson database contains information on 205 different pharmaceuticals. To demonstrate the usability of the Watson database, an exercise was performed to calculate estimates for the year 2015 of concentrations and loads in effluents and removal rates of WWTP's in order to prioritize pharmaceuticals for regulators. Estimates of loads were scaled up to a national level. Furthermore, the potential environmental risk of pharmaceuticals was estimated by dividing the effluent load by the Defined Daily Doses (DDD) of pharmaceuticals as described by the WHO. Due to the overall lack of ecotoxicity data of pharmaceuticals, DDD values may act as an appropriate surrogate as they describe the therapeutic activity and thus general toxicity of pharmaceuticals. The exercise provided valuable information for both water managers and regulators to make cost-effective choices by first tackling those pharmaceuticals with the highest effluent loads and toxicological risks.

MO036

Episode study of pharmaceuticals and personal care products from urban effluents

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Pharmaceuticals and personal care products (PPCPs) as well as their metabolites are contaminants of emerging concern and have been frequently detected in the environment. Their real impact on biota are unknown. Urban effluents are important sources for PPCPs. Their fate and transport in the environment (in particular riverine transport) is not easily traceable since they degrade through both chemical and biochemical reactions and can interact with dissolved and particulate phase in water. Fate and transport are therefore dependent on river characteristics and hydrological regime. It is not clear how extreme high flow events influence emissions and transport of PPCPs. Urban effluents and riverine concentrations from a mid-sized European city (Brno City, Czech Republic) were sampled at high resolution (every two hours) and analyzed for a range of PPCPs during both normal flow and an intense rainfall event. Collected data were used to constrain the hydrobiogeochemical/multimedia contaminant fate model INCA-Contaminants. The model was then used to track PPCPs transport along space and time in the river network.

MO037

Designing a Quality Control Program for Collection and Analysis of Siloxane samples from Environmental Monitoring Programs

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When embarking on an environmental monitoring study, it is critical that the proper quality control (QC) parameters are evaluated to ensure integrity of the results obtained from monitoring activities. This is especially critical when analytes under investigation can be present in personal care products, equipment and analytical instrumentation as is the case for cyclic siloxanes. Selecting an appropriate QC matrix is imperative to accurately account for loss or contamination during collection, transport, and laboratory processing and analysis. In addition, it is important to evaluate analytical variation introduced by the sample matrix itself to the overall signal measured to avoid reporting false positives. It is therefore important that QC matrices be determined prior to the initial design of the monitoring program. Key aspects when designing a QC program include applying an appropriate QC matrix which matches or mimics the sample matrix being collected; determining background contribution from sample collection and laboratory equipment; evaluating initial concentrations present within QC matrices prior to application and how they will evaluate analyte loss or contamination. This presentation will provide an overview of quality control evaluations from several different programs and the best practice for creating a proper QC program. The Loss and contamination of the analyte in the field has been assessed using a variety of materials including fish muscle/liver, sediments and sorbents such as low density polyethylene and XAD® resin. Additionally, processing and analysis QC are assessed to evaluate contamination from equipment such as collection and analysis jars, use of nitrogen as well as analysis equipment.

MO038

Determination of toxic effects of environmental contaminants on gap junctional intercellular communication in TM3 Leydig cell line

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Yawer A., Macíková P., Babica P. Sovadinová I. *RECETOX, Masaryk University, Kamenice 753/5, 625 00, Brno, Czech Republic* Environmental contaminants are substances that, when accidentally or deliberately introduced into the environment, might induce adverse ecological or health effects. Male reproductive system is uniquely vulnerable to such toxic chemicals in the environment. It has been hypothesized that exposures to environmental toxicants is responsible for increasing incidence of congenital malformations of the reproductive organs and testicular cancer, deteriorating semen quality, and decreasing sperm counts, which has been reported in the past decades. Disruptions of hormonal regulation by so-called endocrine disrupting chemicals (EDCs) have been suggested as one of the principal mechanism underlying adverse effects of toxicants on male reproductive system. However, homeostasis balance, morphogenesis, cell differentiation and growth in testicular tissues are physiological processes strictly regulated by gap junctional intercellular communication (GJIC), a process known to be altered by various environmental contaminants, including well-recognized EDCs. We are currently investigating (i) the effects of selected EDCs as well as personal care products (PCPs) on GJIC inhibition in mouse somatic testicular cells, TM3 Leydig cell line, and (ii) which mechanisms are involved in dysregulation of GJIC. The results showed that organochlorine pesticides (methoxycholor, lindane and DDT), and PCPs (triclosan and triclocarban) induced rapid (30 min) dose-dependent inhibition of GJIC in TM3. The effects became more pronounced after 6 or 24 h exposure, where significant reduction of GJIC was induced also by bisphenol A, vinclozolin and PCB153. As determined by Western blotting analyses, the inhibition of GJIC was accompanied by activation of mitogen activated protein kinase ERK1/2, which is the mechanism implicated in GJIC dysregulation induced by known tumor promoters, e.g. 12-O-tetradecanoylphorbol-13-acetate (TPA). Inhibition of GJIC and activation of MAPKs were observed at the concentrations of chemicals not associated with cytotoxic effects as evaluated by Neutral Red uptake, Alamar Blue and CFDA-AM assays. Our results indicate that EDCs and PCPs might disrupt testicular homeostasis and functions via alterations of GJIC and MAPKs.
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MO039

Performing a large scale survey of xenobiotic removal efficiencies of activated sludge treatment plants with a cost-efficient sampling scheme in Luxembourg

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Xenobiotic removal by activated sludge treatment plants can be very variable depending on the design and the operational parameters of the sites. Accurate full scale balances are cumbersome to realize and laboratory sludge experiments have not been related to overall plant performance with enough cases for statistical relevance. Therefore the EmiPoll project has been launched in Luxembourg to evaluate the performance of activated sludge treatment plants with an array of techniques. Full scale plant balances will be performed with passive samplers covering an entire week of inflow vs. outflow. This approach circumvents the problem of mixing regimes and matching volumes in classical autosampler campaigns through the extended time-period of observation. Although for reasons of different suspended matter and DOC loads at the in- and outlet the sampling rates of the passive samplers are different, this can be normalized by conservative substances like f.ex. carabamazepine or lidocaine. The ease of operation allows a multitude of plants to be monitored in parallel with much less workload than autosampler campaigns. The contribution will shed a critical view on the clogging of membranes of passive samplers and the representativeness of sampling in both in- and outlet with this approach. In addition the campaign features laboratory analyses including respirometric tests of the activated sludge, enzyme assays and degradation kinetics of selected substances. The goal is to compare the performance of a large amount of WWTPs (24 sites) and relate the elimination efficiencies with plant design and operational settings. Prioritisation of advanced treatment or leeway in operational improvement will be explored within this project. This contribution presents the results of a first part of the campaign.

MO040
Development of a counter-current chromatography-based extraction method for emerging contaminants
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Complex mixtures of emerging pollutants which include pesticides, biocides, personal care products and pharmaceuticals are constantly present in the hydrosphere and may pose a risk to aquatic ecosystems and human health. The vast and ever-increasing number of chemicals, often present at very low (sub ng/L) concentrations, complicates their monitoring and subsequent regulation. In this context, we propose the application of high performance counter-current chromatography (CCC) as a novel tool for the extraction of a broad variety of pollutants from river waters. CCC is a chromatographic separation technique that has become a novel, worldwide separation and purification technique and is based on the partition of compounds between two immiscible liquid phases as they interact in a thin tube under a fluctuating centrifugal force field. The research explores the use of CCC, not as a separation technique, but as an extraction method for large volumes of water. The extraction is based on partitioning between the two immiscible liquid phases: the mobile phase and the stationary phase. The mobile phase is the water sample potentially containing the pollutants, while the stationary phase is an appropriate organic solvent(s), retained in the column by centrifugal rotation. The advantages of this technique range from little to no sample preparation, and highly versatile solvent systems, to high recovery efficiencies and potentially unlimited extraction capacity. We have applied this powerful tool for the extraction from water samples of a number of compounds, including pesticides, pharmaceuticals, and corrosion inhibitors, covering a wide variety of polarities (log K_{ow} between 1 and 5). Preliminary results demonstrating a range of recovery efficiencies for the majority of these compounds and method development will be presented and discussed.

MO041
The consortia of microorganisms for use in bioremediation
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The continuous development of the industry results in the appearance of chemical compounds in the environment, which do not occur in nature. The different types of them and their metabolites are highly toxic and constitute a threat to humans – some of them are potent poisons, often exhibiting strong mutagenic and carcinogenic properties. Most of the aromatic compounds are characterised by stability and durability in biological systems, and what is more, considerable resistance to degradation. An additional concern is that the contamination of the soil by xenobiotics, is also a direct threat to groundwater, and consequently may result in the penetration of these pollutants into flowing waters. The problem of soil and groundwater contamination by xenobiotics from various branches of industry, concerns not only Poland and other European Union countries, but practically all industrialized world. The main objectives of our study were characterisation of microorganisms (bacteria and fungi) isolated from several environments polluted with petroleum compounds, heavy metals, impregnates for wood and the other xenobiotics. All soil samples were analyzed for the presence of pollutants: heavy metals, PAHs, BTEX, gasoline and mineral oils. The bacterial strains and fungi were identified by PCR amplification and characterised for their ability to degrade certain hazardous substances, for instance mazout, other petroleum compounds and antibiotics in the presence of heavy metals. Assessment of the degradation rate of each pollutant may help to choose those strains, which play the critical role in this process. Selected ones will be part of consortium, which in future might be commercially available as biovaccine. Currently, preliminary studies on this subject has been launched in laboratory systems - microcosms. This approach enable us to test different variants of our study and define optimal relative content of each culture, as well as abiotic conditions. This method in easy and eco-friendly way of removing hazardous pollutants from the environment without introducing any synthetic products into it. The in situ bioremediation is based on natural processes occurring in the environment, which is associated with considerably lower costs than conventional physicochemical methods. The developed and deposited composition of strain (biovaccine) provides a possibility to quickly obtain the appropriate amount of the formulation and conduction of bioremediation in a short time.

MO042
Ecotoxicological endpoints to assess the impact of nanomaterials on soil and water biota
S. Sanchez-Fortun, UCM; G. Costa, Universidad Complutense de Madrid; G. Mengs, Natural Biotec; C. Fajardo; M. Nande, UCM; M. Martin, Universidad Complutense Madrid
Heavy metals are ubiquitous pollutants worldwide. The accumulation of metals in the environment from anthropogenic sources creates the potential for significant human exposures and subsequent health hazards. Deleterious metal-induced health effects have been reported in all body systems, with exposure stemming from multiple sources, including water, air, or soil. Nanotechnology is a rapidly growing area of study with applications in numerous fields because engineered nanoparticles (NPs) unique physical and chemical properties allow them to have higher reactivity than larger particles with the same chemical composition. For the last decade, nanosized zero-valent iron (nZVI) has been used for the remediation of polluted soil, groundwater and wastewater, targeting a wide variety of common environmental contaminants, such as chlorinated organic solvents, organochlorine pesticides, polychlorinated biphenyls, inorganic anions and heavy metals. To this respect, the ecotoxicological assessment of the potential effects of the interaction nZVI-heavy metal on different organisms is an important approach to be considered. Thus, the choice of optimal species as well as sensitive ecotoxicological endpoints may allow the use of shorter testing intervals in the analysis of the interaction NP-heavy metal effects on water and terrestrial organisms. In the present study, we analyze the effects of the interaction NPs (2.5 and 5%) with different concentrations of Cd, Pb and Zn (heavy metals with persistent presence in soil and water) on several species including the green algae *Scenedesmus intermedius* and the cianobacteria *Microcystis aeruginosa*, as representative aquatic biota from fresh ecosystems, and the nematode *Caenorhabditis elegans* as representative terrestrial organism from soil ecosystems. The results in both aquatic and terrestrial organisms showed that selected toxicity endpoints (growth, reproduction, survival and photosynthetic activity) were significantly affected by the heavy metal concentration used. However, these effects were reverted by the presence of nZVI in all cases. Together, these results suggest that: 1) the organisms as well as the endpoints considered have shown to be suitable to analyze the ecotoxicological effects of metal and nZVI interactions, and 2) nZVI have shown to be an excellent method to heavy metal immobilization thus rendering their deleterious effects and making nZVI as an excellent tool in the remediation of contaminated ecosystems.

MO043
THE UKWIR Chemicals Investigation programme Phase 2, 2015-2020
M.J. Gardner, V. Jones, Atkins Ltd; D. Leverett, WCA-Environment Ltd
The UKWIR Chemicals Investigation Programme is the response of the UK water industry to current and emerging legislation on trace substances in surface waters. The first phase of the Programme (CIP1 - 2010-2013) was aimed at the prioritisation of compliance risks posed by different substances and determination of their sources. It provided a generic overview of trace contaminant concentrations in wastewater treatment works effluents and the effectiveness of current treatment processes in reducing contaminant concentrations. The second phase of the CIP is now under way. This £140M programme of monitoring and investigations is focussed on: site-specific compliance status at over 600 wastewater treatment works; the demonstration of where there is a clearly justifiable need for action/investment; and, the potential improvements in effluent quality that might be offered by various novel treatment processes. Participants and contributors include all water companies in England, Wales and Scotland, Defra, Environment Agencies, Ofwat, Atkins Limited, wca-environment and Plymouth and Cranfield Universities. This poster summarises the scope and aims of this ambitious programme and presents some initial results for Water Framework Directive Priority Substances and Specific Pollutants. Results indicate that compliance status might be at risk at the majority of sites for a limited number of specific substances. This implications of this will be discussed.

MO044
Development of a LC/MS/MS method for the comprehensive assessment of of macrolide antibiotics in the aquatic environment
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Macrolide antibiotics are among the most popular types of antimicrobials. As a concequence, a number of studies in the literature addressed the issue of environmental exposure to these emerging contaminants in municipal wastewaters and ambient waters. Macrolide antibiotics are released from the human body after therapeutic application mainly unchanged, however, several literature reports indicated that macrolides can be transformed in the environment either via

microbial or photochemical degradation, yielding a number of transformation products. Nevertheless, most of the existing exposure data refer only to the parent compounds, while the information on possible macrolide intermediates and/or transformation products in the aquatic environment are very scarce. One of reasons is the lack of analytical methods for the comprehensive assesment of macrolide-derived compounds in aqueous samples. Therefore, the aim of this work is to develop a multiresidue method for the simultaneous determination of several prominent macrolide antibiotics (azithromycin, clarithromycin, erythromycin and roxithromycin) along with their intermediates and transformation products using liquid chromatography tandem mass spectrometry (LC/MS/MS). The method development comprised improvement of the chromatographic separation of macrolide compounds using a new type of reversed-phase stationary phase, which combines octadecyl and phenyl moieties, and optimization of enrichment of target compounds from aqueous phase using Oasis HLB and mixed-bed stationary phases. The developed method was employed for the analysis of macrolide-derived residues in real wastewater and ambient water samples, collected in the area of the city o Zagreb. It was shown that intermediates and/or transformation products significantly contribute to the overall mass balance of macrolide-derived compounds. This indicates that the risk associated with the use of macrolide compounds in the aquatic environment should include assessment of possible effects caused by their transformation products and intermediates from their production.

MO045

Comparison of pharmaceutical discharges and treatment from hospital and domestic sources

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Chemical water pollution is more and more studied and documented. Among the most studied pollutants, drugs are special contaminants, due their very low concentrations in the environment and the potential presence of metabolites. Even though effluents from sewage treatment plants are well known to be one of the major sources for introduction of pharmaceuticals into the aquatic system, contributions of significant sources such as hospitals or agriculture are still unclear. Furthermore, despite their high solubility in water, their partition can be significant in the suspended solid phase, although this fraction is not always analysed. In this context, this work consists in a long-term study of hospital and urban effluents and their treatment. Target substances include analgesic, anti-inflammatory, antihypertensive, hormonal contraceptive, antibiotic, anti-fungal, anti-convulsant products and metabolites. Thanks to the use of advanced analytical techniques, limits of quantification consistent with ultra-traces detection were achieved. All the pharmaceuticals analysed were detected. Two molecules, econazole and ethinylestradiol were only detected in the suspended solid phase, which shows the importance of analysing this fraction. Three pharmaceuticals were present at significantly higher concentration in the hospital influent: ketoprofen and two antibiotics: ciprofloxacin and sulfamethoxazole. . Metabolites analysis highlighted the presence of the 4-hydroxy diclofenac and the N4-acetylated sulfamethoxazole at concentrations close to those of the parent molecules. Regarding the water treatment, all pharmaceutical removals were above 50%, except for carbamazepine and diclofenac. Contrary to what was expected, removals were higher with the hospital influent, may be due to the difference of hydraulic residence time. Finally, sludge analysis revealed the presence of eight pharmaceuticals. Among them, three were quantified at concentrations higher than 1 μg/g: diclofenac, ciprofloxacin and more surprisingly salicylic acid, as this molecule is more polar than the others. Thanks to a large number of campaings, this work allowed to acquire consolidated data on the occurrence of pharmaceuticals in hospital and urban wastewaters and their removal. This study also highlights the importance of considering understudied parameters, such as the suspending solid phase and metabolites, for a better understanding of the pharmaceutical fate in the environment.

MO046

Selection of the most environmentally benign ionic liquids option by coupling OECD standardized tests and fish leucocyte mortality

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To reduce health and environmental risks of chemicals and to minimize the environmental footprint of human activities, green chemistry searches for alternative, environment-friendly reaction media and at the same time strives to increase reaction rates and milder reaction conditions. A growing area of research is devoted to ionic liquids (ILs) often termed as "designer" solvents due to the extreme tunability of sought functional properties. As the aftermath of earlier misleading messages reporting ILs generically as intrinsically "safe and risk free" whatever ILs considered, their increase in potential or emerging applications in key domains (like bio-refining, energy storage, nano-material processing, pollution remediation…) dictates a more scientifically-sound approach of actual risk profiles of ILs, including both physico-chemical, health and environmental risks that might be triggered by inadequate selection of use of ionic liquids. In this context, we proposed a advanced approach serving comprehensive evaluation of environmental hazard by combined use of OECD standardized tests (*Daphnia magna* acute immobilization test ; algal growth inhibition test) and fish ex vivo leucocyte mortality analysis. This innovative approach integrating multicriteria assessment has been tested to on a first set of seven imidazolium-based ([BMIM][Cl], [EMIM][Cl], [EMIM][DCA], [EMIM][MeSO3], [EMIM][EtOSO3], [EMIM][BF4], [HMIM][Cl]) and four phosphonium-based ionic liquids ([P6,6,6,14][Cl], [P6,6,6,14][DCA], [P6,6,6,14][(iC8)2PO2], [Pi4,i4,i4,1][TOS]) and is expected to be further used to examine other ILs (biobased, ILs, pyrrolidinium, …) in soon future. Results of ecotoxicity tests obtained so far have revealed a clear potential to rank clearly the families of ILs according to the studied risk and even to identify influencing structural factors within a same family of ILs and compounds within the same family. Complementarity of these tests and ecotoxic properties of IL will be discussed specifically in terms of ecological footprint reduction strategies in chemical engineering processes for successful IL utilization in the future.

MO047

Emerging pollutants in reclaimed water. Is there some concern about crop irrigation?

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Reclaimed water is a strategic effective way to help solve the problem of water scarcity and droughts in the EU. In 2009 in Spain, the current level of water reuse was estimated to be about 500 Mm^3 (Spanish Ministry of the Environment). It is predicted that wastewater reuse in Europe will be 3,222 Mm^3/year by 2025, with Spain in first position (over 1,200 Mm^3/year). Agricultural irrigation is the main application in many EU countries and accounts for around 33% of total water use (EEA, 2012), although this figure comes close to 60% in certain regions of Spain. In this work, reclaimed water samples supplied by a Spanish WWTP were used to analyse the presence of 44 emerging pollutants (pharmaceuticals, parabens and endocrine disruptors). Different microcosms and semi-field studies were performed with three crops (lettuce, corn and radish) irrigated with spring water, reclaimed water and fortified (atenolol, carbamazepine and triclosan) reclaimed water (at 10 and 100 higher levels than the mean observed in reclaimed waters). The aim was to assess possible adverse effects of emerging pollutants on soil-plant systems caused by reclaimed water. The experimental results evidenced no variations in soil functionality, pigments content in leaves or accumulation of emerging pollutants in different plant parts compared to those irrigated with spring water. This work was possible thanks to Spanish Government Grants CTM2013-44986-R and CTM2014-52388-R.

MO048

Human health risk assessment of imidazoliun-based ionic liquids: the role of acetone to their function integrity and toxic profile

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The growing interest for the use of ionic liquids (ILs) in a wide range of applications has raised great concerns about their potential environmental and health hazards. Since ILs, such as 1-butyl-3-methylimidazolium tetrafluoroborate ([bmim][BF_4]) and 1-methyl-3-octylimidazolium tetrafluoroborate ([omim][BF_4]), are widely used either pure or in mixtures with water and/or conventional solvents, such as acetone, the present study investigates their cytotoxic and genotoxic potency in cultured human lymphocytes with the use of cytokinesis block micronucleus (CBMN) assay. Moreover, the interaction of acetone with [bmim][BF_4] and [omim][BF_4] was further evaluated in order to elucidate its role in both ILs function and toxic profile. According to the results, [omim][BF_4]/water (at concentrations ranged from 0.01 to 5 mg L^{-1}) showed increased cytotoxic and genotoxic effects (in terms of % cytostasis and frequencies of micronuclei formation currently measured) on interphase lymphocytes, compared to [bmim][BF_4]/water. In addition, [bmim][BF_4]/acetone (at concentrations ranged from 0.5 to 100 mg L^{-1}) showed increased cytotoxic but not genotoxic levels, compared to those occurred at the respective concentrations of [bmim][BF_4]/water, while [omim][BF_4]/acetone showed a significant decrease of both cytotoxic and genotoxic effects on treated cells, compared to [omim][BF_4]/water. Since [omim][BF_4] appeared to be significantly more cytotoxic and genotoxic than [bmim][BF_4] and showed significant differences in the presence of the carrier solvent (acetone), the possible interactions between acetone and [omim][BF_4] was further investigated. According to preliminary data, interactions of the acetone oxygen atom with the imidazolium ring can be considered. A significant effect of acetone on the interionic interactions and consequently on the ion pair dissociation can also be proposed. Further investigation is in progress. To our knowledge, the present study showed for the first time that the toxic potency of imidazolium-based ILs, such as [omim][BF_4], greatly depend from the carrier organic solvents, such as acetone, thus providing significant information for their development, usage/application and safety.

MO049

Butylparaben and propylparaben: Acute effects on Ceriodaphnia silvestrii

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Emerging contaminants are compounds of natural or anthropogenic origin that can be found in different environmental compartments. They constitute potential risk to biota, affecting the growth and development of organisms exposed to them. Among the compounds classified as emerging contaminants, butilparaben and propylparaben preservatives are often used together in formulations of cosmetic and personal care products, although they may cause several deleterious impacts on biota. Hence, the goal of this study was to evaluate the acute toxic effects of butylparaben and propylparaben, using as test organism the species *Ceriodaphnia silvestrii*, which is a native Brazilian representative of zooplankton. Toxicity tests were carried out using the concentrations: 0.500; 0.750; 1.125; 1.700; 2.550; 3.800 mgL^{-1} of butylparaben and 1.00; 1.50; 2.25; 3.37; 5.00 and 7.60 mgL^{-1} of propylparaben . Acute bioassays lasted 48 hours and they were repeated five times. Results from all acute toxicity tests were subjected to probit analysis using the PriProbit Program V1.6.3 to determine EC_{50} value. The average for the EC_{50}-48h/butylparaben was 1.410 ± 0.024 mg L^{-1} and EC_{50}-48h/propylparaben was 2.98 ± 0.18 mg L^{-1}. The results indicated that *Ceriodaphnia silvestrii* was sensitive to the tested emerging contaminants and the mixture of such compounds at realistic environmental concentrations. Based on this observation and considering that parabens can affect the functioning of several species at even lower EC_{50} values, it can be concluded that additional studies are required to assist the discussions on the implementation of more strict regulations regarding the discharge of these pollutants in the environment.

Flame Retardants: Alternatives, Environmental Fate and Toxicity (P)

MO050

New flame retardants: chemical identification and properties

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Flame retardants (FRs) are chemicals used in a wide range of commercial and household products in order to reduce their flammability. Due to recent legislation and restrictions in the use of brominated FRs, a large number of alternative FRs, mostly non-halogenated, have been introduced to the market. The main aim of this study was to investigate the chemical identity of four new flame retardants technical mixtures and to inform about their properties. For each FR mixture, 5 mg were separately dissolved in iso-octane or methanol, and after appropriate dilution, the solutions were analyzed. A combination of analytical techniques was used, such as gas chromatography-mass spectrometry (in electron impact ionization and electron-capture negative ionization modes) and liquid chromatography-mass spectrometry (with positive and negative electrospray ionization). **CETAFLAM-DB168** (Avocet) is a phosphorus-based flame retardant containing an organic phosphate (tri n-butyl phosphate - TNBP) used for textiles and fabrics in aircraft, automotives, and hotels. It allows durable flame retardant properties to be transferred to polyester yarn and fabrics by simple exhaust techniques. **CETAFLAM-DB9** (Avocet) is a phosphorus-based flame retardant containing a new inorganic phosphinate and an organic phosphate (trichloropropyl phosphate - TCPP) for polyester. It is used in the automobile, clothing, workwear, furnishings, curtains and decoration and public transport textile markets. **RUCO-FLAM PSY-E** (Rudolf Group) is an organic phosphorus flame retardant with high percentage phosphorus content used for polyester (PES) and possibly also polyacrylate (PA) polymers. **PEKOFLAM PES** (Clariant) is an organophosphorus-based non-halogenated flame retardant for use on PES and PA textiles and in many (synthetic) polymer systems with 'wash resistant' effects. It consists of a mixture of cyclic di- and triphosphonates.

MO051

Analysis of organophosphorus flame retardants by GC-MS/MS with EI and APCI ionisation techniques

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Flame retardants (FRs) are human-made chemicals added to consumer and industrial products for the purpose of reducing flammability. Among FRs are the halogenated FRs that may be sub-divided into brominated (BFRs) and chlorinated FRs as well as phosphorus-containing FRs (PFRs). Some halogenated FRs have proven to be persistent, bioaccumulative and toxic to environment, animals and humans. This led governments to adopt restrictions (e.g. for some BFRs). On the other hand, novel brominated flame retardants (NBFRs) and organophosphorus flame retardants (OPFRs) are proposed as alternatives for historical BFRs and their use is in continuous increase. Most OPFRs are introduced as additives and not chemically bound to the polymer; hence they are slowly released in the environment by abrasion and volatilization. Although insufficient toxicity data were available on their toxicity, adverse effects were reported for some of these alternative compounds. The focus of this work was to develop and to optimize an analytical strategy enabling the identification for 18 OPFRs of which 13 are non-halogenated (TEP, TPrP, TiBP, TnBP, TBEP, TEHP, TPP, EHDPP, o-TCP, m-TCP, p-TCP, DPhBP and BPhP) and 5 are halogenated (TCEP, TDCIPP and TCPP for clrinated; TDBPP and TTBNPP for brominated) in fish muscle. The investigated preparation procedure based on pressurised liquid extraction with the in-cell use of Florisil as a first cleanup step (~65% of lipids removal). Gel permeation chromatography was then used as a second cleanup step to maximize the elimination of lipids (~95% of lipids removal). Particular attention was paid to procedural contamination management. Two instrumental methods were developed and optimised in terms of spectrometric and chromatographic conditions by GC-MS/MS operating in positive Electron Impact (EI) or Atmospheric Pressure Chemical Ionization (APCI). The main advantage of 'soft' APCI over 'hard' EI technique was the reduced fragmentation which increases the specificity of transitions in MRM method, especially for the 2 brominated compounds. The perspective of this work will be to evaluate the method performances and hence the reliability of its application.

MO052

Organophosphate Flame Retardants in the Indoor Environment: A Comparison of Central Europe and North America

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Flame retardants (FRs) have been used for several decades to prevent fire and reduce the flammability of a wide range of materials. These properties are offset by their environmental persistency, toxicity and bioaccumulative potential. Worldwide restrictions on the use of old types brominated FRs have therefore resulted in the increased use of alternate compounds, such as organophosphate flame retardants (OPFRs), produced in increasing volumes in recent years. Since in most applications OPFRs are used as additive chemicals and therefore are not covalently bound to the polymeric materials, they can be released easily. Due to their presence in common household products, humans are exposed to the released OPFRs in the indoor environment, especially as people spend most of their time indoors. Air, dust and window film samples were collected from a total of 63 houses and apartments in the Czech Republic, Canada and USA during a sampling period of 28 days in May-August 2013. Samples from one room, usually the bedroom, were collected in each home while a second room, usually the living room, was sampled in 10 randomly chosen homes in each country. In addition to the sampling, data about the building and the household were also collected, i.e. information on electronic equipment and furniture in the sampled rooms, occupants, cleaning, ventilation habits, etc. The objectives of the present study were to examine the concentration of 18 OPFRs found in different matrices in the same room and compare individual rooms in the same home, identify possible sources of these compounds in indoor environments and study regional differences in Central Europe and North America. The OPFRs measured in the highest concentrations were tris(2-butoexyethyl)phosphate (TBOEP), tri-n-butylphosphate (TNBP), triisobutylphosphate (TIBP), tris(1-chloro-2-propyl)phosphate (TCIPP) and 2-ethylhexyl diphenyl phosphate (EHDP) in all countries, however the dominant congeners in each matrix differed slightly between North American and Czech samples. For example, 66% of the total OPFR mass in Canadian air samples was TCIPP and 17% was TNBP, while in Czech air samples 53% was TCIPP but the second highest concentration OPFR was TIBP, accounting for 26% of the total mass. The results suggest there are general similarities in the use and composition of OPFRs between Europe and North America, but differences in individual OPFRs which may be indicative of differences in use between the two continents.

MO053

Halogenated Flame Retardants in Spanish air: a human exposure study.

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Dechloranes (Decs) are flame retardants (FRs) which have been one of the most used chemical families as substitutes of banned FRs such as PBDEs. Chemically, dechloranes are chlorinated norbornene structures. This family include dechlorane 602 (Dec 602), dechlorane 603 (Dec 603), dechlorane 604 (Dec 604) and dechlorane plus (DP). Recently, some studies demonstrated the presence of these substances in atmosphere, environment, biota and even food. Other emerging halogenated FRs that are under study in these works are decabromodiphenyl ethane (DBDPE), pentabromoethylbenzene (PBEB) and hexabromobenzene (HBB). Given that some studies point out the potential bioaccumulative character of these FRs, nowadays their chronical toxicity is a hot issue. However, the evaluation of the exposure and ingestion of these compounds is very important in order to evaluate accurately their toxicity. In this context, our aim was to fill the gap of knowledge about Spanish air concentration of these pollutants and compare it with air concentration of banned PBDEs. To do it, we studied 8 different locations all across Spain. Besides, in one of the biggest cities of Spain, Barcelona, we carried out a study along the week in order to describe weekly trends. Finally, we collected samples of indoor environments from Barcelona to compare indoor and outdoor atmospheres. All these samples (49) were analyzed with a GC-MS/MS technique to determine dechloranes. Besides, the same samples were analyzed by GC-MS to determine PBDEs, DBDPE, PBEB and HBB concentrations. Results showed that PBEB, HBB and PBDE-28 were never detected. From Dec family, Dec 604 was never detected in any sample. The most present pollutants were DP and BDE-209. Range of S_8PBDE in outdoor and indoor environments was 0.36-61.0 and 0.84-18.4 pg/m^3, respectively. Moreover, Decs showed a range from 0.25 to 18.3 and from 3.43 to 27.8 pg/m^3 for outdoor and indoor respectively. With this information, we calculated the human exposure to these pollutants by inhalation and dust consumption. The exposure was evaluated as daily intake (DI). DI of PBDE and Decs in outdoors for an adult were estimated as 16.2 and 0.39 pg/day, respectively. In a work place, the DI for an adult was estimated in 32.2 and 59.6 pg/day for PBDE and Dec, respectively.
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MO054

Distribution of organophosphate ester flame retardants between gas and particle phases - model predictions vs. measured data

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The use of organophosphate esters (OPEs) has increased due to their use as replacements for the restricted polybrominated diphenylethers (PBDEs). That OPEs are a suitable replacement for PBDEs is being debated, especially since recent studies suggest that a number of OPEs have a persistence similar to PBDEs and can be found in remote environments such as the Arctic. The gas-particle partitioning is a key factors to predict where and how chemicals are distributed. The majority of studies measuring OPEs have focussed on the particle- phase, because OPEs have been reported to primarily partition to particles in air. However, considering the variety of physico-chemical properties of OPEs, the expectation that they are mainly in the particle phase "as a class" seems unlikely. To re-assess the hypothesis we compared gas-particle partitioning predictions for 32 OPEs by the commonly used risk assessment tool - OECD P_{OV} and LRTP Screening Tool ("the Tool") – with the partitioning behaviour models of Junge-Pankow and Harner-Bidleman, as well as recently measured data on OPE gas-particle partitioning. The results presented in this study indicated that OPEs with an octanol-air partitioning coefficient (log K_{OA}) below 10 and a subcooled liquid vapour pressure (log P_L) above -5, respectively, partition into the gas-phase rather than the particle phase, regardless of which model was used for prediction. The uncertainty of input data did not change these general observations, even when assuming large uncertainty factors of > 5. The predictions by the Tool as well as the other partitioning models were, furthermore, in good agreement with OPE partitioning measured during a one year stationary sampling by Wolschke et al. (2015). Total suspended particle concentration in air was identified as the factor most influential for the predicted partitioning by the Tool, while the Tool seemed to underestimate the impact of temperature. The generalisation that OPEs as a "class" partition into the particle phase could not be affirmed by the results of this study. Depending on environmental conditions and physico-chemical properties, half of the investigated OPEs were found to primarily partition into the gas phase, as expected from their vapour pressures. Our results emphasise the importance of sampling location and design on measurement results as well as the necessity to assess the risk of OPEs individually, rather than as a homogeneous class.

MO055

Organophosphate esters in the Canadian Arctic - Conclusions from seven years of ship-based observations

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Organophosphate esters (OPEs) have been increasingly used as replacements for the restricted polybrominated diphenylethers (PBDEs) in addition to other uses such as plasticizers. This has led to major increases in environmental levels of OPEs, including the detection in remote environments such as the Arctic where concentrations surpassthose of PBDEs. To assess the behaviour and trends of OPEs concerning long-range transport (LRTP), 14 OPEs were measured in the particle phase of air samples taken during yearly ship-based sampling campaigns from 2007 to 2013 in the Canadian Arctic. In total, 11 out of the 14 OPEs could be detected with highest concentrations and detection frequencies (> 75%) for the chlorinated OPEs TCiPP, TCEP and TDCPP as well as the non-chlorinated OPE TPhP. All other OPEs were detected in 20 % or less of the analysed samples. Median concentrations ranged from 1.0 pg/m^3 (TCP2) to 152 pg/m^3 (TCEP). Interestingly, high concentrations of up to 2300 pg/m^3 were observed for TnBP at a stationary sampling location in Resolute Bay from 2012, while this compound could not be detected in any of the ship-based samples. PIA (version 13) program was used to analyse trends in time-series datasets. We observed general increasing temporal trends from 2010 to 2014 for total OPEs. This was driven by an overall increase of non-halogenated OPEs, while halogenated OPEs seemed to be stable or slightly decreasing. Overall, concentrations of halogenated OPEs seemed to be primarily location and temperature dependent with river discharge from the Nelson and Churchill Rivers (MB) as well as Lake Melville (NF) as sources. In contrast, non-halogenated OPEs seemed to be driven by diffuse sources. The results of this study further emphasise the increasing relevance of halogenated as well as non-halogenated OPEs as contaminants in the Arctic and provides unique information on trends, transportation behaviour and pathways of OPEs into this remote environment.

MO056

Diastereomer profiles of Hexabromocyclododecane in global air samples from the Global Atmospheric Passive Sampling (GAPS) Network

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Hexabromocyclododecane (HBCD) is a globally used brominated flame retardant (BFR), added to Annex A of the United Nations Environment Program (UNEP) Stockholm Convention for Persistent Organic Pollutants (POPs) in 2013. It is a priority chemical for monitoring under Canada's Chemicals Management Plan (CMP) and the Convention's global monitoring plan (GMP). The majority of outdoor air studies have analysed total HBCD content using gas chromatorgarphy mass spectrometry (GC-MS). However determining the contribution of the three main diastereomers present in the HBCD technical formulation, and the three primarily found in the environment: α-, β- and γ-HBCD, may provide insight to the emission sources, transformation and atmospheric transport of the HBCD diastereomers. The present study has developed a sensitive and selective method for the determination of α-, β- and γ-HBCD in outdoor air samples utilising Ultra Performance Liquid Chromatography tandem Mass Spectrometry (UPLC-MS/MS). Polyurethane foam (PUF) disk passive air samples (PAS) from the Global Atmospheric Passive Sampling (GAPS) network, deployed in 2009 and 2014, were analysed with the developed method to provide an indicator of global outdoor air concentrations of HBCD and the relative diastereomer contributions in air. Detection frequency was low, owing to the reduced concentrations of HBCD in outdoor air as compared to indoor air, with HBCD detected in ~20% of the samples and concentrations ranged from < LOD to 7.0 pg/m^3 (∑HBCD).
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MO057

Presence and distribution of 39 emerging flame retardants in a secondary wastewater treatment system

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In this study, an analytical method was developed for analysis of 39 emerging flame retardants (FRs) using a dual instrument platform (LC-MS/MS and GC-(ECNI)MS). Target compounds included: 13 organophosphorous FRs, 7 dechloranes and related FRs, five tribromophenyl ether FRs, 12 monoaromatic FRs and degradation products. The method involved liquid-liquid extraction for aqueous samples and soxhlet extraction for solid and biosolid samples. Extracts for the LC-MS/MS analysis were cleaned up using silica gel and NH2 SPE cartridges. Extracts for the GC-(ECNI)MS were cleaned up using liquid-liquid cleanup with aqueous acetonitrile at three pHs followed by gel permeation cleanup. The methods were validated for accuracy, precision, matrix effects, analyte adsorption as well as sample and extract stability. The developed method was applied to investigate the occurrence and distribution of these flame retardants both in the liquid and solid waste streams of a typical secondary wastewater treatment system employing activated sludge treatment. The method's performance in this study demonstrated its applicability for monitoring these emerging contaminants in the environment.

MO058

Indoor Sources of Tris(chloropropyl) phosphate (TCPP) and Concentrations Outdoors in Tributaries, Rain, and Wastewater Treatment Plant Effluent

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Organophosphate esters (OPE's) are commonly used as plasticizers, lubricants and as flame retardants. OPEs are a concern because of recent reports of high concentrations indoors, in surface waters, herring gull eggs and fish, and their potential toxicity to aquatic biota and humans. Since OPEs are typically employed as additives that are not chemically bonded, they are released from products into the environment via volatilization, dissolution and abrasion. Here we first investigated one indoor source of an OPE and second, documented tris(chloroisopropyl) phosphate (TCPP) concentrations in multiple outdoor media. The indoor source of TCPP to indoor air and dust was linked to spray polyurethane foam (SPF) insulation in a highly insulated home using the isomeric fingerprint signature of TCPP. High levels of TCPP were measured in the highly insulated house in dust (85 ± 47 ug/g) and air (23 ± 8.4 ng/m^3). The isomeric fingerprint of TCPP 1:2 (first and second eluting isomers) were statistically indistinguishable from the SPF insulation used (4.0 ± 0.2, vs 4.1 ± 0.1), whereas the ratios differed significantly from other environmental samples (ANOVA, $p < 0.05$). This evidence suggested that the SPF was the source of TCPP to the highly insulated home. In comparison, the ratio of TCPP 1:2 was 3.2 ± 0.9 in dust from homes in Vancouver Canada and 3.9 ± 0.5 in Istanbul, Turkey. Second, we documented concentrations of TCPP in three Toronto tributaries during high and low flow periods, in the final effluent from three wastewater treatment plants (WWTP), and in bulk rain water. TCPP in WWTPs had the highest average concentrations, followed by tributaries during high flow periods, and tributaries during low flow periods (ANOVA, $p < 0.05$). TCPP in WWTP final effluent was of 6.6 ± 11 µg/L (0.054 -18 µg/L). Tributaries differed from each other only during high versus low flow periods and not from each other, with TCPP concentrations averaging 4.2 ± 3.9 µg/L (0.22-11 µg/L) and 0.86 ± 0.95 µg/L (0.11-6.8 µg/L), respectively. TCPP concentrations in tributaries correlated with water discharge (m3/s), with a Pearson Correlation Coefficient of 0.65. No correlations were evident between TCPP concentrations and suspended solids or turbidity in tributary water, unlike PBDEs. Tributary concentrations of TCPP correlate with other OPEs analyzed in these water, notably TCEP (r= 0.89) and TCDPP (r=0.88).

MO059

Evaluation of sediment contamination by historical POPs and alternative flame retardants in the Gironde estuary

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Human activities exert different types of pressure on ecosystems, including the chemical pressure associated with the release of numerous synthetic organic compounds such as halogenated organics. A number of these chemicals may be persistent, such as polychlorinated biphenyls (PCBs) and polybrominated biphenyls ethers (PBDEs) which were officially classified as persistent organic pollutants (POPs) and are subject to regulations and restrictions. To address these limitations of use, alternative flame retardants (FRs) have been and are increasingly used. A comparison was performed on sediments from the Gironde estuary, between the levels of legacy POPs (PBDEs, PCBs and organochlorine pesticides (OCPs)) and those of emerging FRs: 11 brominated, 7 phosphorus and 6 chlorinated alternative flame retardants including (bis(2-ethyl-1hexyl)tetrabromophthalate (BEHTBP), 2-ethylhexyl 2,3,4,5-tetrabromobenzoate (EHTBB), pentabromotoluene (PBT), tetrabromoethylcyclohexane (abTBECH), octabromotrimethylphenyllindane (OBIND), pentabromoethylbenzene (PBEB), 1,2-bis(2,4,6-tribromophenoxy)ethane (BTBPE), decabromodiphenylethane (DBDPE), hexachlorocyclopentenyl-dibromocyclooctane (HCBDCO), hexabromobenzene (HBB), 2,3-dibromopropyl-2,4,6-tribromophenyl ether (DPTE), tris(2-chloroethyl) phosphate (TCEP), tris(chloropropyl)phosphate (TCPP), 1,3-Dichloro-2-propanol phosphate (TDCPP), Triphenyl phosphate (TPhP), Tricresyl phosphate (TCP), Diphenylcresylphosphate (DCP), Tri-n-butylphosphate (TnBP), Dechlorane Plus (anti, syn), dechlorinated DP products (aCl10DP and aCl11DP), dechloranes (Dec-602 and Dec-603)). Sampling was conducted in September 2014, on 23 points in the subtidal zone and 13 points in the intertidal zone, from the confluence of the Dordogne and the Garonne River to the river mouth at the Pointe du Verdon. This work provides a first mapping of the contamination of the Gironde estuary by legacy POPs and alternative FRs. Controlling factors of POPs/alternative FRs will also be discussed. *Acknowledgements* - The data come from the HALOGIR campaign carried out on the research vessel Côtes de la Manche CNRS-INSU. This study has been carried out in the frame of the Investments for the future Programme, within the Cluster of Excellence COTE (ANR-10-LABX-45). Aquitaine region is acknowledged for PhD grant funding. CPER A2E (Aquitaine region and FEDER) is acknowledged for financial support for instrument acquisition.

MO060

Halogenated flame retardants in eggs of Spanish birds

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Flame retardants (FRs) are compounds applied to materials to increase their fire resistance. Polybromodiphenyl ethers (PBDEs) are the most used FRs and can be found in plastics, furniture or electronic devices. They are classified according to their level of bromination. Since PBDEs are not bonded into plastics but blended, they can leach out and are found in different environmental matrices. PBDEs are persistent and toxic, bioaccumulate and suffer long-range transport. They are considered persistent organic pollutants in the Stockholm Convention and their production has been banned in Europe and North America. Hence new brominated FRs, are used as substitutes; as well as chlorinated FRs, including halogenated norbornenesЙ_or dechloranes. The present study investigates the occurrence of halogenated FRs in unhatched eggs of 7 bird species from central Spain as well as liver tissue from deceased individuals. Samples include 30 eggs and 11 livers from years 2009-2015. The analytical method monitored 8 PBDEs, 3 emerging brominated FRs (DBDPE, HBB and PBEB) and 5 dechloranes (*syn*- and *anti*-Dechlorane Plus and Decs 602, 603 and 604). For the sample preparation lyophilized sample was spiked with internal standards, extracted by PLE, cleaned and purified by an acidic attack and SPE. Extracts were analysed by GC-MS/MS. Method recoveries for the compounds ranged 51-88 %. Method LODs and LOQs were 0.01-3.2 ng/g lipid weight (lw) and 0.03-10.8 ng/g lw. PBDEs were detected in all samples, with total concentrations below 319 ng/g lw, except for one sampling spot where they reached 4,292 ng/g lw. BDE-47, -99 and -100, which correspond to the PentaBDE commercial mixture, were the main contributors to the profiles. Dechlorane levels were in the same order of magnitude as PBDE levels in all the spots. Even without taking the most polluted spot into account, the rest of the results are still higher than those found in Doñana Natural Park (south of Spain), maybe because the latter is better protected [1]. *Acknowledgments* – This work has been funded by the Generalitat de Catalunya (Consolidated Research Group Water and Soil Quality Unit 2009-SGR- 965). [1] E. Barón, M. Máñez, A. C. Andreu, F. Sergio, F. Hiraldo, E. Eljarrat, D. Barceló. 2014. First report of pyrethroid accumulation in river fish: A case study in Iberian river basins (Spain). Environ Int 68: 118-126.

MO061

Occurrence of polybrominated diphenyl ethers and emerging halogenated flame retardants in food items

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Brominated flame retardants (BFRs) are chemicals used in a wide range of commercial and household products in order to reduce their flammability. Because most BFRs are not chemically bonded to the products which they are added to, they can easily leach into the environment. People are mainly exposed to BFRs via dust and diet. In particular, the lack of data on the occurrence of BFRs in food prevents an accurate estimation of the human intake. The main aim of this project was to follow up the European Commission Recommendation 2014/118/EU on the monitoring of BFRs in various food items on the Belgian market and to provide data on their occurrence and levels in the main food categories. The presence of polybrominated diphenyl ethers (PBDEs), novel BFRs (hexabromobenzene (HBB), bis(tribromophenoxy)ethane (BTBPE), tetrabromobenzoate (TBB), tetrabromophthalate (TBPH)), tribromoanisole (TBA), and dechlorane plus (syn-DP and anti-DP) in various food items was assessed using an analytical method developed and validated in this project. This novel method uses low sample amounts and solvent volumes, and involves a two-step clean-up, including florisil and acid silica. The determination of BFRs was performed using gas chromatography-mass spectrometry operated in electron-capture negative ionization mode (GC-ECNI/MS). The validated procedure was first used for the determination of BFRs in 20 individual food items purchased from Antwerp supermarkets (including fish, meat, chicken eggs, cow milk, grains and vegetable oil), and, in a second stage, has been used for the analysis of more than 200 food samples in the frame of the Belgian food monitoring project. A high extraction performance was obtained, which, in combination with an efficient two-step clean-up procedure, enabled the accurate determination and quantification of the target compounds in a wide variety of food matrices, with different characteristics and lipid content. In all food categories, PBDEs were the most frequently detected and were predominant in fish/seafood samples. Among other FRs, TBA was detected only in fish/seafood, indicating its primary natural origin from the marine environment. For the other compounds, the results showed a generally low contamination of all analyzed food samples, with values generally below the quantitation limit (LOQ).

MO062

Brominated flame retardants in Belgian foodstuffs - recent evaluation by a novel UPLC-MS/MS method

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The study was undertaken as consequence of the Commission Recommendation 2014/118/EU on the Europe-wide monitoring of brominated flame retardants (BFRs) in food. BFRs are anthropogenic chemicals that are added to a wide variety of consumer products in order to improve their fire resistance. BFRs may leach from the products into the environment. Due to their persistence and potential to bioaccumulate in the food chain, BFRs may cause toxic effects in humans and animals. There is a lack of information on the occurrence of BFRs in foodstuffs, which has hampered accurate completion of intake assessments. The main objective of this work was to evaluate the presence and to measure the levels of BFRs, namely brominated phenols (BPs), hexabromocyclododecanes (HBCDs), tetrabromobisphenol A (TBBPA) and its derivatives in foodstuffs consumed by the Belgian population. Quantitative measurements were performed using ultra-high performance – tandem mass spectrometry (UPLC-MS/MS) on an ACQUITY UPLC system coupled to a Xevo-TQ-S mass spectrometer. Sample preparation protocol consisted of a QuEChERS-based extraction followed by two parallel clean-up procedures. Column chromatography with acidified silica gel and dispersive solid-phase extraction with C18 and carbon sorbents were used to eliminate lipids, pigments and eventually other matrix components from the extract. The method is applicable to a wide variety of food matrices and was in-house validated. Representative portions of food samples belonging to different categories, such as fish and seafood, meat and meat products, chicken eggs, oils and fats, milk and dairy products, were collected from Belgian (super)markets in 2015. The samples were pooled per food category according to the consumption data of the Belgian Food Consumption Survey, after which they were lyophilized and subjected to the multi-analyte LC-MS/MS analysis. In this work, data on the occurrence and levels of BFRs in different food commodities consumed in Belgium will be presented. The results will be discussed with regards to the frequency of occurrence and highest detected concentrations of the BFRs, and compared to existent data for other (European) countries. This data will be decisive in the subsequent calculation of intake levels. *Acknowledgments*: Financial support from Federal Public Service (FPS) Health, Food Chain Safety and Environment

MO063

Gas chromatography/atmospheric pressure chemical ionisation/tandem mass spectrometry to perform non-polar brominated flame retardants monitoring in food items at ppt levels

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Brominated Flame Retardants (BFRs) have been analysed in food items for a decade in France to establish the French food exposure. The analytical methodology was first focused on Polybrominated diphenyl ethers (PBDEs) often implying a double injection on a gas chromatograph coupled to a high resolution mass spectrometer in electromagnetic field to detect the low and high m/z separately in HRSIM, i.e. from tri- to hepta-BDE in the first injection and deca-BDE in the second one. Since some PBDEs products have been replaced by novel or emerging BFRs, the list of compounds of concern in food is still increasing continuously (118/2014/EU recommendation), adding one or two more injections of the same extract in GC/HRMS and increasing the analysis time and the cost of each analysis dramatically. Nowadays, analytical alternatives are envisaged such as gas chromatography coupled with tandem mass spectrometry using atmospheric pressure chemical ionization (GC/APCI/MS/MS), which allow a higher throughput analysis. The aim of our work was to propose a methodology to monitor PBDEs and 12 additional BFRs under "routine conditions", i.e. a single injection of the extract conducted in a short acquisition time under robust conditions. The interface parameters were first optimized and the APCI+ ionization under dry mode was chosen with a 2 μA corona current. Then, MS/MS events were mainly performed on the molecular ion, giving a high specificity to the signal. The quality of BFRs introduction in the mass spectrometer being the most critical point, the inlet conditions were completely reviewed. The chromatographic system was optimized in working on the fundamentals, i.e. injector and liner qualities, columns geometries and associated velocities and gradient of temperature (always on 5MS stationary phase type). A compromise was established on the basis of the optimal HETP and resolution calculation and on the DecaBDE degradation to maintain at the same time the integrity of heaviest BFRs and chromatographic efficacy and resolution for the other ones. The final methodology was implemented on the national control plan samples and illustrations will be given during the SETAC conference to describe? the robustness of the GC/APCI/MS/MS method and its applicability for routine analysis at ppt level in food.

MO064

In vitro bioaccessibility of plasticisers present in indoor dust using simulated human lung fluids

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Plasticizers are additives imparting durability, elasticity and flexibility in the manufacture of polymeric products such as PVC flooring, retailing and packaging materials. The lack of migration stability has resulted into their classification as major indoor contaminants. Despite their extensive everyday use, the process of assessing human exposure and possible health effects arising from indoor air contamination only began the past decade with limited results so far. This study forms a part of the EU Marie Curie Initial Training Network "Advanced Tools for Exposure Assessment and Biomonitoring" (A-TEAM) aiming to develop and establish novel methods on human exposure biomonitoring of pseudo-POPs including phthalate esters (PEs) and alternative plasticisers, having as a study group indoor dust originating from N=61 participants' houses in Norway. In the present study, we investigate the *in vitro* bioaccessibility (i.e. uptake/absorption) of plasticisers present in indoor dust collected from vacuum cleaner bags with respect to potential routes of exposure including inhalation and respiration. Simulated artificial human body fluids are used regarding the relevant exposure route, i.e. lung fluids. Indoor dust is sieved at 63 µm using an methanol-washed metallic sieve. 200 mg of sieved dust samples are incubated with 20 mL of artificial lung fluid (ALF) at 37ºC for the artificial lung fluid in order to set up a realistic exposure scenario. All media incubations are conducted continuously for 96h. After the incubation step has finished, the samples are centrifuged at 3000rpm for 10min and the supernatant is subjected for a liquid-liquid extraction (LLE) using MTBE:Hexane 3:1. A selective and sensitive method to determine the analytes of interest is employed by using triple quadrupole gas chromatography mass spectrometry (GC-MS/MS) in multiple reaction monitoring (MRM) mode. The method validation was performed using Standard Reference Material for indoor house dust (i.e. SRM 2585) in replicate analysis and the results show that low molecular weight phthalates (e.g. DMP & DEP) are more than 85% bioaccessible through the simulated lung fluids, while the high molecular weight phthalates and alternative plastisisers (e.g. DEHP and DEHT) presented bioaccessibility values less than 5%.

MO065

Are some "safer alternatives" of Flame Retardants hazardous as PBTs? Screening of FRs by the cumulative PBT Index in QSARINS

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Flame Retardants (FR) are chemicals widely used to enhance fire safety of industrial and commercial products. However chemicals like PBDE, largely used in the past, demonstrated to have unintended negative effects to human health and wildlife due to their properties of Persistence, Bioaccumulation and Toxicity (PBT). Nowadays such chemicals are replaced by New FRs (NFRs), as New Brominated FRs (NBFRs) and Halogen Free FRs (HFFRs) or Organophosphorous FRs (OPFRs) supposed to be "safer alternatives". Nevertheless, the information about the chemical properties of these substitutes are often not available and these substances were commercialized without complete information regarding their PBT properties, that are based on long-term behaviors and would require complex and expensive experiments. However, the PBT assessment is expressly required in the context of REACH regulation and PBT chemicals require an authorization. In recent years many monitoring studies have identified these alternative FRs in various environmental compartments and in wildlife. In this study, several new compounds, proposed and used as "safer alternatives" to PBDEs, were analyzed with multivariate evaluation tools and screened with the cumulative PBT Index, a MLR QSAR model that allows to identify PBT chemicals directly from their molecular structure, implemented in QSARINS (QSAR INSubria) software. A rigorous check of the chemicals that are included in the model Applicability Domain (AD), and for this reason with the most reliable predictions, has been done. The results, obtained directly from the chemical structure for the three properties altogether, have been compared with those obtained by the US-EPA PBT Profiler and a good agreement (73%) between the two different approaches was found, resulting in a more precautionary assignment of PBT Index. A priority list of the potentially most hazardous FRs, predicted by consensus from both the approaches, has been proposed, highlighting that some compounds, proposed and already used in commerce as supposed safer alternatives, are identified as intrinsically hazardous for their PBT properties, explaining why they are detected all over the world. This study also shows that stuctural modeling approaches, such as PBT Index, could be valid tools to evaluate appropriate and safer substitutes, *a priori* from the chemical design, in a benign by design approach, avoiding unnecessary synthesis and expensive experimental tests.

MO066

Identification of species differences in the effects of androgenic compounds using in silico and in vitro methods

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Endocrine disrupting chemicals (EDCs) are compounds that cause reproductive and developmental disturbances. Brominated flame-retardants (BFRs) are one group of EDCs that have been shown to affect the normal function of endocrine, behavioural, reproductive, metabolic and neurological systems. BFRs are used extensively in commercial and industrial appliances to meet fire safety regulations and reduce the flammability of materials. Because of their low cost and high efficiency the use of BFRs has increased rapidly. These, in turn have resulted in an increased release to the environment and these have been detected in avian, aquatic and mammalian species. In this study we included two groups of BFRs, TBECH diastereomers (α, β, γ and δ) and derivatives of 2,4,6, tribromophenol (TBP), allyl 2,4,6-tribromophenyl ether (ATE), 2-bromoallyl 2,4,6-tribromophenyl ether (BATE) and 2,3-dibromopropyl 2,4,6-tribromophenyl ether (DPTE) that were studied for their effects on androgen receptor (AR) functioning. To investigate whether the TBECH diastereomers and TBP derivatives bind to the androgen receptors of the chicken, zebrafish and human an *in silico* molecular docking and dynamics procedure was performed by using the MOE and ICM modelling software. These showed that both sets of BFRs can bind to the ARs in the different species. To confirm the *in silico* results, *in vitro* activation assay studies by using chicken LMH, zebrafish ZFL and human HepG2 cells were performed. This demonstrated that the TBECH diastereomers acted as agonists and the TBP derivatives acted as antagonists to the ARs. It also showed that TBECH diastereomers are strong inducers of the human AR followed by the zebrafish and chicken ARs. This reveals that because of the sequence difference between the species the same compound can function differently. The TBP derivatives set acted as strong antagonists with comparable potencies between the species. In conclusion, the majority of chemicals that are being used in products have still not been tested and their effects on the endocrine system have therefore not been studied. The rapid identification of compounds that can disrupt the normal functioning of the endocrine system is crucial and this study demonstrates that the use of *in silico* and *in vitro* techniques can be used as a fast and reliable method of identifying compounds with unknown biological properties.

MO067

Applying the RAIDAR model for ecological risk assessment: A case study for 10 organic flame retardants.

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Chemicals are being evaluated to determine if they pose unacceptable risks to humans or the environment. Measured (monitored) concentrations in environmental media are non-existent or limited for the vast majority of chemicals produced and used by society. The extensive exposure data gaps hinder the application of risk-based methods for chemical prioritization, screening and comprehensive assessments. The chemical activity approach has been proposed as an integrating concept for chemical risk assessment. The Risk Assessment Identification And Ranking (RAIDAR) model includes multimedia mass balance fate calculations for organic chemicals in air, water, soil, sediment and representative aquatic and terrestrial species and food web bioaccumulation models comprising various trophic levels (e.g., plants, invertebrates, fish, birds and mammals). Here we conduct a case study for screening-level ecological risk assessment using measured and estimated exposure and toxicity data and the RAIDAR model for 10 organic flame retardants (OFRs). The 10 OFRs include chlorinated, brominated and organophosphate flame retardants. The chemicals cover a diverse range of chemical properties for partitioning, reaction, persistence, bioaccumulation and toxicity. A database of 3,120 measured concentrations of 10 OFRs in temperate North America is used to derive emission rate estimates using inverse modelling and to evaluate the model calculations. Estimates of risk are quantified by comparing exposures and effects expressed in terms of chemical activity. Uncertainty in model input parameters (partitioning properties, degradation half-lives, emission rate estimates and toxicity) is used to estimate uncertainty in the RAIDAR risk calculations. A comparative risk assessment is used to rank the OFRs for their relative risks to the environment based on current information for toxicity and chemical emission rates. Comparable risks of OFRs in the environment are partially explained by an inverse relationship between chemical emission rates and overall persistence.

MO068

Toxicity Profiling Of Flame Retardants In Zebrafish Embryos Using A Battery Of Assays

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The developing zebrafish embryo is increasingly used as an alternative animal model for evaluating toxicities. The aim of this study was to assess the toxicity of 10 flame retardants (FRs) of environmental concern using a battery of zebrafish toxicity assays. With the phasing out of brominated FRs (BFR), organophosphate FRs (OPFRs) are increasingly being used. However, there is limited information on their potential for inducing adverse health effects. In this blinded study, 2 BFRs and 8 OPFRs (6 non-halogenated, 2 halogenated) were tested for developmental toxicity, cardiotoxicity, behavioral alterations, and hepatotoxicity. A bioavailability study was conducted to measure the internal compound exposure by LC/MS/MS. To determine the risk of teratogenicity of the tested compounds, the presence of morphological and functional alterations were analyzed and a teratogenic index (TI) established as the ratio of the LC50/EC50. High TI values were obtained for 3 compounds - isopropylated triphenyl phosphate (IPP), tert-butylphenyl diphenyl phosphate (BPDP), and tricresyl phosphate (TCP) (EC50 ~2-5 μM), - that highlight the possible teratogenic potential of these compounds. In terms of cardiotoxicity, 4 of the 6 non-halogenated OPFRs tested (IPP at 100 μM, BPDP from 10 μM, TCP from 30 μM, and triphenyl phosphate from 10 μM) induced bradycardia followed by auricular failure, a specific and uncommon zebrafish cardiotoxic effect. 2,2'4,4'-tetrabromodiphenyl ether (BDE-47) specifically affected locomotor activity from 1 μM, concentration at which not other deficits were observed. Finally, hepatotoxicity was found after the treatment with 2-ethylhexyl diphenyl phosphate (EHDP) and BPDP, for both at 10 μM. In conclusion, the OPFR results support the need for more extensive testing and demonstrate the applicability of this battery of zebrafish assays. This abstract does not necessarily reflect the views of NTP/NIEHS.

Microplastics in the environment: Sources, Fate and Effects (P)

MO069

Comparison of different procedures for quantifying microplastic particles in freshwater samples

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(Micro)Plastics litter is one of the main environmental issues of the 21st century. While marine litter has been recognized by politics (e.g. through MSFD) and society as an important problem requiring the need for action, the knowledge regarding its origins is still limited. There is for example no information on the amount of litter being transported through rivers into marine habitats. Research institutes as well as authorities have recently started to quantify riverine (micro)plastic litter, but this is being done with a range of different methodologies and approaches. On this account no comparative studies or comparable assessments can be conducted. This project aims at comparing and evaluating five different approaches for quantifying microplastic particles in freshwater samples. Water samples from the river Rhine, Germany, were taken using an Apstein plankton net (150 μm mesh size). Water is eliminated via filtration with membrane glass fiber filters, lyophilisation or centrifugation in three of five procedures. Afterwards, there is an enzymatic digestion of organic compounds using cellulase (E.C. 3.2.1.4), chitinase (E.C. 3.2.1.14) and lipase (E.C. 3.1.1.3) in the first step, followed by addition of protease (E.C. unknown) after some incubation time. In the end a density separation using sodium chloride is performed. The fourth procedure does not feature removal of water before the enzymatic digestion. The last procedure begins with a density separation and filtration ahead of the digestion. Recovery experiments are done using four different kinds of colored plastics: polypropylene (PP), polystyrene (PS), high-density polyethylene (PE-HD) and low-density polyethylene (PE-LD). The particles were categorized into three groups of 200 μm – 630 μm (30 particles of each material), 630 μm – 2000 μm (20 particles) and 2000 μm – 4000 μm (3 particles). After going through the different procedures the remaining particles were counted using a digital microscope. First results of our current research indicate that the recovery and feasibility of the procedure employing water removal via freeze-drying is most successful. A gravimetric comparison concerning the effectivity of the enzymatic digestion of the filtered and freeze-dried sample is ongoing and therefore further results will be presented at the conference.

MO070

Canada's regulatory approach for microbeads

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Microbeads are released into the environment from a variety of applications, including consumer and industrial products. To evaluate the potential environmental impacts of microbeads, the Government of Canada in 2015 completed a science summary, which involved the review of >130 relevant publications and reports. Based on the findings from the review, it was recommended that microbeads be added to the list of toxic substances under the Canadian Environmental Protection, 1999. In addition, a mandatory information gathering survey was published for microbeads to gather sector specific data along with a notice of intent to develop regulations for microbeads. This presentation will highlight key findings from the science summary.

MO071
Fate and impact of model microplastics in freshwater
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Plastics, originating from voluntary or involuntary release, are considered as an emerging contaminants of global importance and rise concerns about their environmental implication. Although the environmental impact of macroplastic waste is extensively studied, the behavior and the effects of micro-plastics, either unintentionally released in the environment, either formed as a degradation product of the macroplastics is still largely unknown. Owning to their small sizes, microplastics can be readily ingested by the aquatic microorganisms, affect them and accumulate in the aquatic food chain. The present study therefore explores the fate of microplastics in surface water and their effect to water flea *Daphnia magna*. The stability of two model plastic nanoparticles with similar primary size of 200 nm and different surface functionalization, amidine- and carboxyl- group functionalized latex beads, were characterized following their hydrodynamic size and surface charge variations in lake and river water, and *D. magna* test medium. Furthermore the effect on *D. magna* was quantified by using 48h exposure immobilization test and following the uptake in the gut by microscopic observations. Negatively charged carboxyl-latex beads were stable in all tested media as revealed by the unchanged negative z-potential and lack of agglomeration. Interestingly the behavior of the amidine-latex beads was strongly affected by the exposure medium conditions characterized by strong agglomeration in lake and river water, however were stable in *D. magna* exposure medium. Both the positively and negatively charged latex particles were consumed by zooplankton. The amount of the microplastic particles detected in the *D. magna* gut increase with their concentration in the exposure media. The 48h-immobilization tests demonstrated toxicity both micro-plastic particles to the water flea. The EC_{50} of 50 mg/L and 80 mg/L for amidine- and carboxyl- latex beads, respectively were found demonstrating that these microplastics can be considered as harmful for *D. magna*. Overall the obtained results demonstrated the potential of the microplastics to affect aquatic biota, as well as the importance of the particle surface charge in particle-biota interactions and in their reactivity and stability in the aquatic systems.

MO072
The fate of microplastics ingested by Antarctic krill (Euphausia superba)
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Plastics particles less than 5mm (microplastics) are generally accepted to be ubiquitous within the marine environment. With the concentrations of marine microplastics increasing worldwide and reaching the most remote ecosystems, it has become necessary to develop a greater understand of how marine species interact with this relatively recent addition to the marine environment. Antarctic krill are a key species in the Antarctic ecosystem. They are undoubtedly one of the most important organisms in the southern ocean as almost all species residing in the Antarctic depend on krill either directly or indirectly. They also exhibit great dietary flexibility which may leave them vulnerable to the adverse effects of plastic ingestion, including false satiation, leaching of chemical additives and reduced body condition. In order to determine the capacity of Antarctic krill to ingest microplastics and the fate of ingested particles, krill were fed a diet of microplastics and algae with a range of concentrations. The diet consisted of 10, 20, 40 and 80% plastic per dry weight of algae, which ranged from approximately 14.5 to 116.2 plastics mL^{-1}. The model microplastics were fluorescent polyethylene beads with a size range of 27-32 μm and density of 1.026g/cm^3. Krill were kept in 24hr darkness in 0°C filtered seawater for ten days. Feeding occurred daily for four hours and water changes occurred before and after feeding. Faecal pellets were found to contain whole beads and small fragments of plastics, indicating that the mechanical forces of the gastric mill were able to triturate microplastics particles. To investigate this finding further, histological analysis was performed on the digestive tract and on whole krill. The findings are outlined in this presentation.

MO073
Occurrence of microplastics in the stomach content of red mullet (Mullus barbatus) from the Western Mediterranean
C. Martinez-Gomez, A. Martinez camara, V.M. Leon, Instituto Español de Oceanografia / Marine Contamination and Biological Effects; D. Vethaak, DELTARES / Marine and Coastal systems
In this study the occurrence of microplastics (MPs) (< 5 mm – 300 μm) in the stomach content of red mullet (*Mullus barbatus*) from three areas along the Spanish Mediterranean coast was investigated. Potential relationships between MP ingestion and physiological and reproductive parameters in this species were also investigated. Fish stomachs were collected during routine monitoring programs for chemical contamination in 2013 (fixed in 10% *formalin* followed by storage in 95% *alcohol) and 2014* (frozen at -20° C). Making use of alkaline digestion methodology (KOH 1M, 21 days, ambient temperature) and microscope-based counting (stereo microscope) it was found that percentage of fish with MPs in its stomach was 24%, with a predominance of fibers (66%) and soft spheres (33%), representing between 1 and 8% of the total stomach content weight. We found no relationship between plastic abundance in the stomachs and gonadosomatic index and condition factor of the fish (p> 0.05). Our preliminary results generally agree with several previous investigations on plastic content in fish stomach. The identity of the removed particles and fibers await further confirmation using Fourier transform infrared (FTIR) spectrometry. These are the first observations on ingested plastic in a benthic fish species from the Western Mediterranean Sea, contributing to the knowledge about the magnitude of this environmental problem. **Acknowledgement:** This work has been supported by IMPACTA project and by the CleanSea project, part of the European Union Seventh Framework Programme (FP7/2007-2013), under grant agreement n° 308370.

MO074
An Optimised Extraction Method of Fluoranthene from Microplastics using Accelerated Solvent Extraction
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Microplastics (MPs) are a group of anthropogenic contaminants with a high persistence in the natural environment. MPs that became brittle or fragmented may release plastic additives like plasticizers, which are known to have toxic effects on the biosphere. In addition, hydrophobic toxic pollutants like polychlorinated biphenyl (PCBs) or polycyclic aromatic hydrocarbons (PAHs) in the aquatic environment may be accumulated on the surface of MPs. In order to determine the ecotoxicological impact of MPs, it is necessary to understand the nature and extent of the chemicals that travel a long distance with MPs in the environment. However, currently no harmonized analytical methods are available to extract MPs from the environmental matrix as well as to extract pollutants from the MPs. Here, we propose a reliable method to determine the mass of contaminants adsorbed onto MP using an accelerated solvent extractor (ASE). First, a series of batch experiments were carried out in the laboratory to charge fluoranthene, a 4-ring PAH, onto plastic pellets (ID < 5 mm) made of three different polymers: polyethylene (PE), polystyrene (PS) and polypropylene (PP). Then, fluoranthene adsorbed on the pellets was extracted using an ASE ® 300 (Dionex, Idstein) under a fixed pressure of 100 bar. The ASE extraction was carried out either in one or two cycles (approx. 5 minutes per cycle) at 50, 70 and 100°C depending on the physicochemical persistence of the pellets. Methanol and isopropanol were tested as solvents in this study. The ASE conditions were optimized for the extraction of fluoranthene on the surface of PE-, PS- and PP-pellets to yield a high recovery rate. First results showed that the best ASE extraction conditions for PE were at 100° C for 5 minutes in two static cycles using isopropanol, leading to an extraction efficiency of approx. 70 -80%. The similar extraction efficiency was determined for PS at 70°C, which is the maximum possible temperature for the pellets, for 5 minutes in two static cycles using isopropanol. The highest recovery rate of 110 -115% was obtained for PP under the ASE condition at 100°C in 1 static cycle using isopropanol.

MO075
Alternative pathways of microplastic exposure - microplastics and associated POPs on zebrafish gills (Danio rerio) and zebrafish eggs
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We here present an alternative pathway for the transfer of microplastics and associated pollutants by exposing zebrafish (*Danio rerio*) gills and eggs to very small microplastic particles (1-20 μm) loaded with the polycyclic aromatic hydrocarbon (PAH) benzo[a]pyrene (BaP). So far, microplastics and the transfer of associated pollutants has mainly been studied by the ingestion of microplastic particles into various organisms. Potential effects of microplastics *via* adherence to and accumulation on gills has only been studied in bivalves and crustaceans, however without analyzing the transfer of adsorbed substances. Likewise, effects by microplastics, as well as potential accumulation and transfer of pollutants to fish eggs has been neglected. Adult zebrafish were exposed to 1-5 and 10-20 μm

microparticles loaded with BaP for 6 and 24 h and analyzed for effects by both virgin and BaP-loaded microparticles on gills in the ethoxyresorufin-*O*-deethylase assay (EROD) on gills and visual fluorescence tracking of BaP. Similarly, zebrafish eggs were incubated for 96 hpf with virgin and BaP-loaded microparticles and analyzed *via* the fish embryo toxicity test (OECD 236), visual fluorescence tracking of BaP after 48, 72 and 96 hpf and the *in vivo* EROD assay after 96 hpf. Results show that microparticles mostly adhere to the mucus of fish gills and are thus subsequently washed out during exposure times. Nevertheless, there was a trend to EROD induction in gills of fish groups exposed to BaP-loaded microparticles. The fluorescence analyses located BaP on gill arches rather than on filaments, if compared to positive controls. In contrast to fish gills, microparticles readily accumulated on the chorion of fish embryos. Fish embryos showed sublethal effects following exposure to BaP-loaded microparticles, whilst virgin microplastic particles had no effect. The present study illustrates that microplastics do not only cause effects by intestinal ingestion, but also by adherence to surface structures such as gills and the egg shell. This represents an alternative exposure route to food-borne consumption of microplastics and associated pollutants.

MO076

Adsorption of four endocrine disrupting compounds on polyethylene microplastics

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Scientific studies have shown that plastics greatly contribute to the littering of the environment. Micro-sized plastics, termed as microplastics (MPPs, bisphenol A, estradiol, dienestrol, hexestrol) on polyethylene MPPs (40~48 µm) was investigated. Solution parameter effects of pH and natural organic matter (NOM) were also considered. Our results demonstrate that the adsorption isotherms of all the pollutants to MPPs complied well with the langmuir model. Q_e values decreased with pH increase (5~10), mainly due to the different speciation of compounds under different pH values. It is noteworthy that an overall trend of higher Q_e values increased with NOM increase, probably due to covalent effects.This result also implies that the presence of NOM may increase the contaminants' burden on MPPs, and subsequently increase MPPs' carrier potential for contaminants to aquatic organisms and lead to greater risks to the ecological system.

MO077

Microplastics effects and bioavailability of co-contaminants in the blue mussel Mytilus edulis

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Microplastics (5 mm–1 µm) are the most numerous pieces of plastics reported in marine environments and concerns exist regarding their potential to affect organisms. Potential negative effects of microplastics (MPs) include disruption of gut physiology after ingestion, release of substances (co-contaminants) sorbed to MPs into organisms, and occlusion of tissue surfaces by accumulation of MPs. Mussels are particularly at risk as they filter large volumes of water and have potential to accumulate MPs on gills and/or within the gut lumen.

Co-contaminants, such as polycyclic aromatic hydrocarbons (PAHs) and metals, can sorb to MPs and desorption after MP ingestion is of toxicological concern. The objective of this study was to assess the bioavailability of associated co-contaminants to MPs in mussels, as well as to study MPs physical effects. *Mytilus edulis* mussels (one per beaker) were exposed for 24h to polyvinyl chloride (PVC) pristine particles (125-250 µm diameter), at 10 different concentrations (0-1 g/L). Co-contaminants, cadmium (Cd^{2+}) and the PAHs anthracene and pyrene, were allowed to be sorbed to MPs (>24h) prior to exposure to mussels. A dose-response according to concentration of co-contaminant while in aqueous phase was also established for mussels. Gene expression was assessed in gills and digestive gland using pi-Glutathione S-transferase (*pi-GST*) as a biomarker of PAHs bioavailability and Metallothionein-20 (*MT-20*) for Cd^{2+}. Gene expression was measured using quantitative reverse transcription PCR (RT-qPCR) after RNA extraction from gills and digestive gland. Samples of the same tissues were also placed in Davidson's fixative, sectioned and analysed to assess lesions and inflammatory responses. Preliminary results indicate that Cd^{2+} sorbed to PVC particles induced *MT-20* expression in the digestive gland of *M. edulis*: up to 5 fold change compared to control, a value within a similar range in mussels exposed to Cd^{2+} in aqueous phase (3.26 fold change in *MT-20* expression). The expression of *MT-20* increased linearly with increased particle concentration in the digestive gland, as for mussels exposed to Cd^{2+} in aqueous phase, but not in gills. Males that spawned during the experimental procedure did not exhibit *MT-20* expression in digestive gland tissues. We anticipate that our results, together with ongoing data analysis, will contribute to a better understanding of the fate and effects of MPs and their co-contaminants in the marine environment.

MO078

Microplastic may increase uptake and bioaccumulation of organic pollutants in aquatic crustaceans

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Microplastic is causing an increasing environmental problem in aquatic environments where it has been shown to enter into the food chain. Maybe even more important, microplastic may act as a contaminant trap and carrier of organic pollutants that could enhance bioaccumulation of these compounds in aquatic food chains. The uptake of microplastic in *Moina salina* was investigated using different concentrations and size fractions of microplastic particles. The amount of plastic taken up by the animals was determined by microscopy and the sublethal effects of microplastic on *Moina salina* were determined using video tracking and enzymatic assays. *Moina saliina* ingested microplastic particles at various rates in these experiments. Microplastic particles similar to the ones used for the feeding experiments were allowed to adsorb 14C-phenantrene, before these were fed to *Moina salina*. The radioactivity of the animals and of the microplastic particles was measured by liquid scintillation counting. The data obtained from these experiments were used to determine to which extent microplastic might act as a carrier of organic pollutants in aquatic environments.

MO079

Microplastics: Investigation of different effects on sorption behaviour of organic pollutants in freshwater systems

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Nowadays synthetic polymers are an essential part of everyday life and an introduction of synthetic materials in the environment cannot be completely avoided. One part of this discharge are microplastic (MP) particles with diameters ≤5 mm. Due to a slow degradation, MP, as well as macroscopic plastic, has a high impact on ecosystems. Freshwater systems are a revealing field of research, as these systems play an important transport route of MP in sediments and oceans world-wide. Many aspects of the influence of MP on the environment are still under investigation and thus their effects on ecosystems are yet not fully understood. One aspect of this research is the determination of sorption properties of MP, which are based on different polymer materials such as polyethylene (PE), polypropylene (PP), polystyrene (PS), polyvinylchloride (PVC) and polyethylene terephthalate (PET). Environmental organic pollutants such as pesticides or pharmaceuticals can act as sorbate and accumulate onto MP due to hydrophobic interactions. Thus, MP may provide a transportation route for organic pollutants in aquatic systems or the food chain. Sorption processes for various organic pollutants are still under research and seem to be dependent on various parameters such as polymer material, age of the particles, biofilm formation and further environmental parameters. The aim of this study was to evaluate different synthetic polymers regarding their potential to act as sorbent for organic pollutants. Therefore, synthetic polymers were investigated in synthetic fresh water that was spiked with different micropollutants. The utilized polymer materials were polybutadiene, PS and PVC, which were each spiked with pesticides such as e.g. difenacoum and bromadiolon. The MP samples were extracted with hexane or dissolved in tetrahydrofuran and underwent purification via gel permeation chromatography and/or solid phase extraction before the content of organic pollutant was determined via GC-MS or LC-MS/MS. All polymers showed pesticide sorption and sorption isotherms were calculated for the different polymer/pesticide combinations applying *Henry, Freundlich* and *Langmuir* models. As the sorption isotherms were determined for an ideal system (synthetic polymers, synthetic fresh water), the importance and influence of further parameters have to be determined, such as the formation of biofilms and "old" MP samples.

MO080

Ingestion of environmental microplastics causes biomarker responses and behavioral changes in three-spined stickleback

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Microplastics (MPs) raise great environmental concerns due to the wide distribution in aquatic environment and dreadful capacity to absorb and concentrate environmental contaminants. As potential threats of MPs are greatly unknown, interdisciplinary research is being accelerated towards increasing understanding of biological fate of MPs in organisms, their toxicity and inherent ecological consequences. An experimental study was conducted to study vector effects of MPs following environmentally relevant exposure scenarios. Unplasticized polyvinyl chloride (uPVC) microscopic pellets were used as model particles, which were exposed for 30 days to the effluent of sewage treatment plant and the outflow from industrial harbor in Göteborg city, Sweden. Consequently, marine adult three-spine stickleback *Gasterosteus aculeatus* were feed diets containing exposed particles. Fish were fed with experimental diets for

24 days and then potential chemical uptake and toxicity was assessed using gene and biochemical biomarkers. Additionally, behavior of exposed fish was investigated with open-field and aggression tests. Results revealed that environmental MPs can cause changes in mRNA expression levels of several genes in liver, for example, expression of metallothionein (MT) a known biomarker for metal toxicity and vittelogenin (VTG) an established biomarker for estrogenic exposure. Some changes in behavior were also observed. Further investigations are conducted to fully assess the dietary toxicity of MPs and to elucidate the composition of environmental pollutants adsorbed to MPs.

MO081

PET microplastics have no long-term effects on the freshwater amphipod Gammarus pulex

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Microplastics (MP) are abundant in most of the global marine and freshwater ecosystems and can interfere with its biota. Potential adverse effects have already been studied *in situ* as well as in laboratory studies for several marine species while effects on freshwater organisms remain so far largely unstudied. The present study provides first results on the short-term uptake and the long-term effects of PET MP on the freshwater amphipod *Gammarus pulex*. As part of an uptake study (24 hours) as well as an effect study (48 days) juvenile (6-8 mm) and adult (12-17 mm) individuals were exposed to fluorescent PET MP with a size range of 10-150 μm. Both studies covered MP concentrations from 0.4-4,000 particles per ml. The rate of MP uptake throughout 24 hours was determined by enzymatically lysing the exposed individuals and analyzing the ingested particles with a fluorescent microscope. Feeding activity, energy reserves (glycogen, lipids, proteins), moult periods and mortality were investigated in the 48-days chronic toxicity study. The results of the short-term uptake study indicate that MP ingestion by *G. pulex* is not size-selective and increases with the particle concentration. Comparing the absolute and relative uptake of adults and juveniles at same particle exposure concentrations, juveniles ingested more particles than adult individuals. In the chronic toxicity study no significant changes in feeding activity, energy reserves, moult period duration and mortality were observed in any of the treatment groups. In conclusion, this study demonstrates that a common freshwater amphipod readily ingests MP. However, the uptake does not result in adverse effects on behavioral, physiological and developmental parameters. The absence of long-term effects might be the result of the main feeding strategy of amphipods. *G. pulex* shredders detritus and plant materials and is adapted to a frequent uptake and egestion of non-digestible particles. Future studies will provide insight on whether MP adversely affect freshwater species with other feeding strategies (e.g. filter feeders).

MO082

Uptake and toxicity of methylmethacrylate-based nanoplastic particles in aquatic organisms

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The uptake and toxicity of two poly(methylmethacrylate)-based plastic nanoparticles (PNPs) with different surface chemistries (medium and hydrophobic) was assessed using aquatic organisms selected for their relevance based on the environmental behaviour of the PNPs. Pure poly(methylmethacrylate) (medium; PMMA PNPs) and poly(methylmethacrylate-*co*-stearylmethacrylate) copolymer (hydrophobic; PMMA-PSMA PNPs) of 86-125 nm were synthesised using a mini emulsion polymerisation method. Fluorescent analogues of each PNP (FPNPs) were also synthesised using monomer 7-[4-(trifluoromethyl)coumarin]acrylamide and studied. *Daphnia magna*, *Corophium volutator* and *Vibrio fischeri* were employed in a series of standard acute ecotoxicity tests, being exposed to the PNPs at three different environmentally realistic concentrations (0.01, 0.1, and 1.0 mg L^{-1}) and a high concentration 500-1000 mg L^{-1}. In addition, sublethal effects of PNPs in *C. volutator* were determined using a sediment reburial test whilst the uptake and depuration of FPNPs was studied in *D. magna*. The PNPs and FPNPs did not exhibit any observable toxicity at concentrations up to 500-1000 mg L^{-1} in any of the tests except for PMMA-PSMA PNPs and FPNPs following 48 h exposure to *D. magna* (EC50 values of 879 and 887 mg L^{-1}, respectively). No significant differences were observed between labelled and non-labelled PNPs, indicating the suitability of using fluorescent labelling for tracing of the NPs. Significant uptake and rapid excretion of the FPNPs was observed in *D. magna*.

MO083

Effects of microplastic presence on PBDE bioaccumulation and mortality of the freshwater invertebrates Chironomus sancticaroli and Lymnaea stagnalis

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Studies have shown that microplastic particles in the environment can associate with persistent organic pollutants (POPs) due to the hydrophobic nature of plastics and organic chemicals. This is likely the case wherever microplastics are present, given that microplastics in the environment will rarely exist in the absence of other environmental pollutants. If ingested, the gut environment of an organism may favour desorption of these adsorbed chemicals due to gut condition including pH and ionic strength and the presence of surfactants. Therefore ingestion of microplastic particles has implications for uptake and bioaccumulation of these chemicals. PBDEs (polybrominated diphenyl ethers) are widely used as flame-retardants in products such as textiles and soft furnishings, with the potential to leach into the environment, so are one of the likely POPs to find associated with microplastics. We conducted bioassays using freshwater midge larvae *Chironomus sancticaroli* and the pond snail *Lymnaea stagnalis* to observe the effects of microplastic particles (nylon < 50 μm) and PBDEs both independently and in combination, including mortality, effects on the gut microbiome, stress gene expression and PDBE bioaccumulation. Microplastic particles were mixed with quartz sand sediment at 1% concentration (13g total substrate per replicate). A PBDE mix (containing BDE-47, 99, 100, 153 and PBB-153) was added to the sediment-microplastic mix in glass vessels at six environmentally relevant concentrations (94, 188, 375, 750, 1500, 3000 ng g^{-1}). Artificial freshwater was added to each vessel once the solvent had evaporated. Given that POPs will preferentially bind to plastics over sediment, it was assumed that all PBDEs would adsorb to the nylon particles rather than the sediment or the glass vessel. Exposures ran for 96 hours during which there was no mortality effect on either species. Following the exposures RNA, microbiome DNA and tissue chemical analyses were carried out. A parallel study showed ingestion of microplastics by chironomids over a 48 hour period, with microplastics still present in the gut following a 48 hour depuration period, demonstrating that microplastics are ingested and retained. Similar observations have been made on microplastics within a pond snail gut. The results of this study will help develop our understanding of the interactions between microplastics, organic chemicals and organisms in the environment and the implications for the food chain.

MO084

Influence of microplastics on the short-term toxicity of mercury to juveniles of the sea bass (Dicentrarchus labrax)

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The pollution of the marine environment by microplastics is global paradigm. More knowledge on the toxic effects of microplastics alone and in mixture with other common global pollutants such as mercury are need to support environmental risk assessment of these chemicals. The objective of the present study was to investigate the influence of the presence of microplastics in the water on the short-term toxicity of mercury to juveniles of the European seabass (*Dicentrarchus labrax L.*) After acclimatization in laboratory conditions, groups of juveniles were exposed to mercury and microplastics (polyethylene microspheres 1-5 μm), alone and in mixture for 96h under controled conditions of temperature and photoperiod. Control was filtered sea water; two concentrations of mercury and two concentrations of microplastics were tested in a full factorial experimental design. Effect criteria were swimming performance and sub-individual biomarkers indicative of neurotoxicity, alterations in biotransformation, energy production, and anti-oxidant defences, and lipid peroxidation damage. For several parameters, including the swimming velocity, significant differences among treatments were found, with fish exposed to mercury alone and in the presence of microplastics showing a reduction of the predatory performance ($p < 0.05$), and impairment of several important physiological functions ($p < 0.05$). Moreover, for some of the parameters, interactions between microplastics and mercury were found. These findings highlight the need of more research on the interactions between microplastics and other common contaminants on marine organisms.

MO085

Effects of microplastics on benthic macroinvertebrates in freshwater ecosystems

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Recently, the presence of microplastics (MPs) in the freshwater environment has been reported and little is known about the effects these MPs can have on freshwater organisms. It is expected that sediment-dwelling species are especially susceptible to the presence of MPs in freshwater ecosystems, as sediments could act as a sink for MPs. Previous studies done on the marine environment already showed that MPs can have an effect on benthic species, such as the reduction of fitness in the lugworm *Arenicola marina* (L.). Therefore, the aim of this study is to investigate the effects of MPs on species which are suitable for representing the freshwater benthic community. For this purpose, single-species toxicity tests were performed, where species were exposed for 28 days to 8 different concentrations of polystyrene (PS) beads with a size range of ~40-90µm. The concentrations tested in this study ranged from 0.1% to 20% (in order of percent dry weight), including the highest concentration of MPs found in the freshwater environment, which is ~1%. The dose-response relationships were calculated for each species using growth/weight as a sub-lethal endpoint and survival as a lethal endpoint. This data will be used to elaborate a Species Sensitivity Distribution (SSD), showing the differences in susceptibility for MPs between species. The SSD can also be used in the future to determine the maximum allowable concentration of MPs in the freshwater environment.

MO086
Decreased feeding or food assimilation is a likely physiological mechanism of microplastic effects in the filter-feeding crustacean Daphnia magna
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Marine and freshwater contamination with microplastic (MP) has received increasing focus during recent years. For example ingestion of MP by various filter-feeding organisms including the freshwater cladoceran *Daphnia magna* has been documented. However, we still have limited knowledge of MP effects in freshwater environments. In theory MPs may affect exposed organisms by four different mechanisms: 1) ingestion of MPs decrease food intake by interfering with feeding or filling up the gut, 2) MPs may in other ways interfere mechanically with the physiology of the organisms, 3) toxic additives (e.g., plasticizers) may leach from ingested particles, 4) environmental chemicals may adhere to particles leading to changed (i.e., increased or decreased) uptake of the chemical in question. The purpose of the present study was to test the hypothesis that MPs interfere with feeding or food assimilation leading to effects at the organism level and eventually to potential population-level effects. Two experiments were carried out according to a slightly modified *D. magna* reproduction test (OECD 211). Experiment 1 tested potential effects of particle size (1-10 and 10-106 μm), using 0.96 g/cc polyethylene (PE) beads. Experiment 2 tested effects of particle density using PE beads in the same size range (10-90 μm) but with different densities (ca. 1.00 and 1.10 g/cc). In both experiments survival, growth and reproduction was measured during a 21 days study. The study was a proof-of-principle study using particle concentrations (i.e., 1000 and 10,000 particles/ml) above likely realistic environmental concentrations. There were no significant effects of MP exposure on *D. magna* survival regardless of particle size or density. Growth (after day 15) and reproductive output were decreased significantly by the MP particles with the highest density, but not by any of the other particle types. The fit of a dynamic energy budget model indicated that the observed effect was caused by either a reduction in feeding or food assimilation or by increased maintenance cost. The study shows that impacts of plastics depend crucially (though not surprisingly) on plastic properties such as density, and that certain properties (e.g., size) are unimportant if particles end up in a different environmental compartment than the organism. The next step should be to test other combinations of particle size, density and potentially shape at environmentally realistic particle concentrations.

MO087
Microplastics in rivers: Focus on short-term temporal and spatial variabilities
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Microplastics in freshwater is an increasing issue. These plastics defined as a particle with a size smaller than 5 mm have been widely reported in marine environments. This study aims at investigating the homogenity of microplastic distribution in rivers and also to evaluate the short tem and spatial varaibility of microplastics in rivers. The Marne river was considered. The samplings were performed with a plankton net (mesh size 80 μm). Two separate campaigns were carried out in order to assess the short-term variability of the microplastic concentrations. Six samples were collected per campaign. On the first campaign, each sampling was performed during 1 minute while it was performed during 3 minutes on the second campaign. For the variability through the cross section, triplicates in five different points were sampled. The sampling duration was 3 minutes. Three points of sampling were on the surface with one in the right bank, one in the left bank and one in the center. The two remaining points were in the center with respectively 1 and 2 meters of depth. The depth of the water at the moment of the sampling was 2.6 m. During short-term temporal variability tests, concentrations ranged between 38.2 and 101.6 particles/m^3 in the first campaign with a coefficient of variation around 45% and between 18.7 and 38.6 particles/m^3 during the second with a coefficient of variation of 26%. The water flow was also different. In fact, the second campaign was carried out after a month of particularly dry weather leading to a very low water flow. Microplastic concentrations seem to be more variable in high water flow conditions. A lateral variability (n=9) of 53% is found. The higher concentrations observed near the banks might be related to the effect of the intense fluvial traffic in the Parisian agglomeration. In fact, it can expected that a barge passing generates waves that drive microplastics and floating debris towards the banks. Considering all samples collected in the middle of the river, a coefficient of variation of 21% (n=9) is found which shows that the vertical variability is more than two times lower than the lateral variability. This result is not surprising knowing that in river conditions, a constant water mixing is induced by the currents and reduces the chances of stratification. Moreover, the passing of the barges can easily contribute to this mixing.

MO088
Microscopical investigations of polystyrene microparticles colonization by Vibrio crassostreae
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Microplastics represent a widespread contamination in the global environment, particularly documented for the oceans. Recent studies of microorganisms' diversity associated with microplastics show a large presence of bacteria of the genus *Vibrio*. Particularly, some species pathogenic for marine organisms were identified associated to microplastics collected at sea. These discoveries raise questions about the potential role of microplastics as a vector for pathogenic organisms in the marine environment. A protocol to study the colonization of polystyrene (PS) in controlled conditions was designed, with the aim to understand factors modulating the adhesion on these microparticles. The pathogenic bacteria *Vibrio crassostreae* J2-9 was used in this study owing to its pathogenicity for oysters *Crassostrea gigas*, and constitutive mutants expressing GFP (Green Fluorescent Protein) were constructed to follow the colonization using fluorescent microscopy. Colonization processes and dynamics were investigated *via* confocal fluorescent microscopy and scanning electron microscopy for different types of polystyrene microparticles (smooth, irregular, fluorescent and non-fluorescent) over 24h to 96h. Incubations were performed in different media to assess the influence of some abiotic (presence of nutrients) and biotic (presence of multi-species microorganisms associated with natural seawater) parameters. The results of this study highlight complex processes of colonization/decolonization: for all particles types the percentage of colonised particles was higher in a Zobell culture media rich in nutrients as compared with filtered synthetic seawater. Roughness of the particles led to a better colonization than what was observed for smooth PS microbeads. In natural seawater, presence of natural agregates formed by debris and microorganisms around the particles led to a strong and long lasting colonization, suggesting that *V. crassostreae* could play a role as a second colonizer in the marine environment. The knowledge developed during this study opens the way for further studies addressing this topical issue. **Key words**: microplastics, polystyrene, *Vibrio,* colonization

MO089
The effects of microplastic on fresh water Hydra attenuata morphology & feeding
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Microplastic refers to small pieces of plastic Hydra attenuata. Microplastics (polyethylene) used were sourced by vacuum filtering a face wash product containing microbeads. *Hydra* were then exposed to different concentrations of these microplastics (Control, 0.01, 0.02, 0.04, 0.08 g/ml) and morphology was recorded at 24, 48, 96 hrs and 1 week. Feeding tests were also carried out using the same concentrations as the exposure by recording the amount of *Artemia* ingested over a period 120 min. *Hydra* were found to be able to ingest the microplastics (22 - 444 μm) and feeding was affected by the presence of microplastics even at the lowest concentration used (0.01 g/ml). After 60 min of exposure the *Hydra* ingested a lower amount of *Artemia* at all concentrations of microplastic compared to the control. This became particularly evident at the 90 and 120 min mark. Exposure to the microplastics did cause changes to the morphology of the *Hydra*, however these changes were non-lethal. The preliminary results of this study show that *Hydra attenuata* are capable of ingesting microplastics. The presence of microplastics disrupted the feeding behaviour of the *Hydra* thus negatively impacting its ability to feed. Continued research in this area will provide valuable insight in determining the potential impact microplastic can have on the health of freshwater biota.

MO090
Quantification of microplastics emissions from cosmetic products to freshwater ecosystems
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Recently, a lot of attention has been put on small pieces of plastic materials

commonly called “microplastics”. There are two sources of microplastics; microplastics generated through fragmentation of larger plastic material called secondary microplastics and primary microplastics – particles manufactured at a specific size range usually used as abrazives (microbeads) in cosmetic products. After usage of these products, microbeads are released into sewage and travel across wastewater treatment plant and are finally discharged into surface waters. However, the amount of microbeads released into the environment has not been yet assessed. Hence, the aim of our study was to estimate amount of microbeads that can daily be released from cosmetic products to surface waters. The study initially characterized the microbeads from cosmetic products with an emphasis on physical properties and particle size distribution and consequently quantified the daily emission of microbeads from consumers by a questionnaire survey. Amount of microbeads in five selected scrubs varied across products, but the highest amount of microbeads was 11% of the cosmetic product. All microbeads were made of polyethylene with the major particle size up to 100 μm. Survey participants (n =178) were estimated to release 15 mg of microbeads per person per day. If wastewater treatment plant efficiency (~ 70% microbeads removal) is taken into account the daily emission of microbeads into surface waters is 4.5 mg of microbeads per person being potentially more than 2 tons of microbeads per day released in the European Union. Results of our study showed, that microbeads from cosmetic products can represent a potential threat for freshwater ecosystems, because they are continuously introduced into surface waters and due to their small size (up to 100 μm), they can be easily ingested by a range of aquatic organisms.

Behavior Revised: Examining Behavioral Effects of Contaminants and Other Stressors in Aquatic Animals (P)

MO091

Impact of the antidiabetic drug metformin on brown trout

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With a focus on two groups of emerging micropollutants in aquatic environments, food additives and pharmaceuticals, the project Eff-Net crosses the borders between (natural and socio-political) science and public interest by providing an analytical network, which gives insight into the cross-linking of consumer behaviour and ecological impact. In a multi-disciplinary approach, antidiabetics (e.g. metformin), artificial sweeteners (e.g. sucralose) and antidepressants (e.g. fluoxetine) as well as their metabolites and transformation products are studied from the mode of action at the molecular level up to the effects at the community level. In the present study, we investigate possible effects of metformin, an antidiabetic drug, in brown trout (*Salmo trutta* f. *fario*). The influence of different temperatures is also addressed. Starting with the exposure of fertilized eggs, developing eggs and juvenile fish are continuously exposed to different concentrations of metformin (1x, 10x, 100x and 1000x the environmental concentration). The experiment is conducted at two different temperatures, regarding possible interactions of chemical toxicity and temperature. To show influences on embryo development, mortality, hatching success, and time of hatch are recorded. At the end of the sac-fry stage, several endpoints indicating fish health are investigated: these include the histological condition of liver, glycogen storage in this metabolic organ and alterations in the level of the stress protein Hsp70. Additionally, animal behaviour (swimming velocity and predator-prey-interaction) is investigated. The information gained from this experiment at the organismic level is used to validate results of experiments addressing effects at the molecular and community level (generated in parallel by other partners of Eff-Net) with respect to their ecological relevance.

MO092

Sublethal effects of the combined exposure to metal mixtures and predator stress on Asellus aquaticus

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In aquatic ecosystems, trace metals often occur in mixtures in which they can strongly interact with each other, creating synergistic, additive or antagonistic toxic effects. Moreover, natural stressors such as temperature fluctuations or predators are present, which can increase the animals' vulnerability to these contaminants. However, classic toxicity tests are performed using single compounds without additional stressors and could thus lead to an over- or underestimation of the impact of metal exposure in the natural environment. This study investigates the effects of the combined exposure to predator stress and metal mixtures of copper, cadmium and lead on the freshwater isopod *Asellus aquaticus*. The chosen metal concentrations are equal to and tenfold of the environmental quality standards (EQS): 7 and 70 $\mu g.L^{-1}$ for Cu; 0.15 and 1.5 $\mu g.L^{-1}$ for Cd; and 7.2 and 72 $\mu g.L^{-1}$ for Pb. Animals were exposed to the different metals separately as well as to binary and tertiary mixtures. These metal treatments were combined with two predator treatments in which cues of both fish and invertebrate predators were absent or present. After ten days, various sublethal endpoints were assessed. Besides determining the metal accumulation and the growth rate of each animal, we mainly focused on behavioral endpoints such as swimming performance and feeding rate. Furthermore, the energy reserves (glycogen, protein and lipid concentrations), oxidative stress (SOD, CAT and TBARS) and respiration were measured. We hypothesized that metal accumulation, respiration and oxidative stress would be higher in the animals of the (metal) treatments with predator stress. However, we expected the animals of the treatments without predator cues to have higher feeding and growth rates, higher energy reserves and to be more active. This study will help to understand how anthropogenic and natural stressors interact and contribute to the development of ecologically relevant EQS.

MO093

Sublethal Effects of Glyphosate and Glyphosate-Copper Complexes to Daphnia magna Determined by Video Tracking and Behavior Analysis

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Glyphosate (N-(phosphonomethyl)glycine) is the active ingredient in a range of popular broad-spectrum herbicide formulations. Glyphosate is a chelating agent (ligand), and can form complexes with divalent metal ions including copper (Cu). The toxicity of glyphosate to non-target organisms has been evaluated in previous studies, however, little is known about the bioavailability and ecotoxicity of glyphosate:copper complexes to non-target organisms. We have used video tracking to quantify behavioral changes in *Daphnia magna* after exposure to glyphosate and glyphosate:Cu(II) complexes. Behavioral responses were quantified for individual juvenile *D. magna* after exposure for 24 h and 48 h. Sublethal concentrations of glyphosate:Cu (II) resulted in decreases in swimming velocity, acceleration speed, and distance moved whereas the inactive time of *D. magna* increased. Active/inactive time and distance moved were the most responsive parameters to glyphosate:Cu(II) exposure. On a molar basis, glyphosate:Cu(II) complexes were more harmful to *D. magna* than glyphosate alone. The EC50-48 h for a 1:1 mixture of glyphosate and Cu(II) was 13.8 μM and 9.8 μM determined as change in active time and distance moved, respectively. In comparison, visual observation of immobility resulted in an EC50-48h of 25.5 μM. The video tracking results indicated that exposure of *D. magna* to binary mixtures of glyphosate and Cu(II) attenuated acute metal toxicity but increased apparent glyphosate toxicity due to complexation. The results suggest that glyphosate is a likely mediator of environmental metal toxicity, and that video tracking provides an opportunity for quantitative studies of sublethal pesticide effects to non-target organisms.

MO094

Are we mainly looking at the bright side of life? The role of food as an exposure pathway

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The ecotoxicological testing of chemicals is mainly focusing on waterborne exposure pathways. Considering the physicochemical properties but also the mode of action of some chemicals, however, their impact via the ingestion of contaminated food or their implications for the nutritious quality of food may have serious influences on the behavior and physiology of aquatic organisms. We will demonstrate that chemicals with a high affinity towards organic carbon (i.e., lambda-cyhalothrin) adsorb to food, which ultimately potentiates the effects caused by the waterborne effect pathway. Moreover, systemic pesticides such as neonicotinoids but also insecticidal proteins, produced by genetically modified plants, showed a higher toxic effect when organisms fed on related plant material compared to the water-borne toxicity of leached chemicals. This suggests that the compounds are bioavailable to the test species during gut passage, a process not reflected in standard toxicity tests. Moreover, substances targeting autotrophic (e.g., herbicides) or heterotrophic microorganisms (e.g., antibiotics and (in)organic fungicides) can affect the quality of the food for the next trophic level, namely grazing and leaf shredding invertebrates. Experimental efforts have largely focused on heterotrophic microorganisms and shredders. These data suggest on the one hand that shredders can sense toxic effects on heterotrophic biofilms associated with organic matter because they showed preferential feeding on non-contaminated food with a presumably higher nutritious quality. On the other hand, the organisms’ energy processing and physiological fitness can be substantially influenced when dependent on food with a modified heterotrophic biofilm. This may ultimately cause bottom up directed effects in heterotrophic food webs. We discuss the relevance of the food-related exposure pathway with respect to empirical evidence and the broader ecological context.

MO095

Spontaneous Locomotor Changes in Zebrafish (Danio rerio) when chronically exposed to Chemical Warfare Agents (CWAs) found in the Baltic

Sea.

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After the 2nd World War the CWAs were prohibited by law and 11,000 tonnes of toxic agents were dumped in the Bornholm Basin east of Bornholm. The dumped chemical munitions have not reached attention from politicians and scientists until recently. During earlier projects, such as MERCW (2005-2008) and CHEMSEA (2011-2014), the area has been screened for the presence of parent compounds and metabolites including the concentrations they are found in. The majority of the detected compounds has been found in the sediment and a minor part in the pore water. The (eco)toxicity of these compounds remain to be illuminated in which this study hopefully will contribute to. Especially, chronic toxicity needs to be described as this mimics a more environmentally realistic situation. One or two compounds will be accessed based upon various factors such as detection frequencies, found concentrations in both sediment and pore water, acute toxicity and physicochemical properties. Besides the mentioned evaluation factors, Sulphur mustard (Yperite) degradation products will have emphasis as the majority of the dumped CWAs is the sulphur mustard gas. The chronic toxicity will be described by spontaneous locomotor changes in Zebrafish (*Danio rerio*). The CTDB database and Zebrafish gene collection reveals that genes associated with locomotion interacts with some of the found parent and degradation compounds of CWAs; even though the locomotion is a complex out of an array of genes. This study intends to draw lines to the commercially important cod (*Gadus morrhua*). The cod migrates down to the seafloor – even crossing the oxycline - where the CWA munitions were dumped. To sum up, this study will obtain novel ecotoxicity data on recently discovered degradation products and assess the potential threat to the commercially important cod (*Gadus morrhua*). This study is a part of the NATO Science for Peace and Security (SPS) project "Towards the Monitoring of Dumped Munitions Threat" (MODUM).

MO096

Waterborne toxicity and altered food quality affect the food-processing behavior and physiology of Asellus aquaticus - implications for food webs in streams

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The breakdown of terrestrially derived leaf litter is a key ecosystem process for energy provisioning in headwater streams mediated by microbial decomposers and detritivorous, leaf-shredding macroinvertebrates. Stressors like fungicides, however, can impact the functions of these decomposer-detritivore systems via indirect and direct pathways. To disentangle the relevance of each pathway, a 2x2-factorial test design was applied: leaf litter was conditioned under exposure to the model fungicide epoxiconazole (0 and 15 μg/L) and fed to the shredder *Asellus aquaticus*, kept under control conditions or exposed to the same epoxiconazole concentration over 28 days. Endpoints were chosen related to the conditioning status of leaf material as well as the food-processing and physiology of *A. aquaticus*. When fed with fungicide-affected leaf litter, *Asellus* increased consumption (5%) and depleted excretion (20%) compared to the control, indicating compensatory feeding and enhanced utilization of the ingested food. This behavior may have been triggered by a lower leaf-associated microbial biomass (fungi: 25%; bacteria: 15%) after conditioning under fungicide exposure. Reduced growth (30%) and lipid content (8%) of asselids, however, suggest these processes to be insufficient to fully compensate for the energy requirements. Hence, dietary fatty acids (FA) essential for asselids' growth might have been additionally used as an energy source, given the lower essential FA contents (10%) compared to control organisms despite a higher availability of FA on the leaf material. Waterborne exposure led to an increased assimilation of the ingested food, judged by lowered consumption (5%) and excretion (10%). Due to reduced growth (37%) and lipid content (21%), this compensational mechanism was judged insufficient, as it induced a mobilization of internal energy reserves to cope with chemical stress. For the combined (worst-case) scenario an additive action of both pathways was found: compared to the control, asselids' consumption and excretion were reduced by 17% and 15%, respectively. Similarly, depletions were found for growth (44%) and lipid content (28%). These results suggest that fungicides have the potential to affect the ecosystem functioning of decomposer-detritivore systems via several pathways. A more comprehensive understanding of each pathway's relevance seems therefore fundamental to accurately predict effects on energy provisioning in heterotrophic stream food webs.

MO097

Migratory behavior, metabolism, oxidative stress and mercury concentrations in marine and estuarine European glass eels (Anguilla anguilla)

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This study investigate the relationships between the estuarine migratory behavior, Methylmercury (MeHg) concentrations, oxidative stress response and detoxification processes in glass eels, collected in marine and estuarine waters at the end of the fishing season (April). Glass eels migratory behavior was investigated in an experimental flume according to their response to dusk. Fish responding to the decrease in light intensity by ascending in the water column and moving with or against the flow were considered as having a high propensity to migrate (migrant). Glass eels still sheltering at the end of the 24 hrs catching period were considered as having a low propensity to migrate and were called non-migrant. Our results provide some evidences that estuarine glass eels present a higher propensity to migrate and lower indications of oxidative stress than marine glass eels. This might reflect a selection process, non migrant marine glass eels progressively settling in the estuary and/or a change in feeding behaviour. Indeed, in April, glass eels restart feeding in the estuary which might decrease the oxidative stress related to starvation, and enhanced migration. There was no difference in MeHg concentrations between migrant and non migrant glass eels. However, it is suggested that non migrant glass eels might present a higher vulnerability to stress (at least contamination and/or starvation), although the underlying mechanisms remain to be elucidated.

MO098

Behaviour as a Tool to Assess the Effects of Environmental Contaminants in Crustaceans: A Review

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Behavioural effects have been used in toxicology testing since the 1960s as they provide a link between biochemical and ecological consequences of environmental contaminants. A review was conducted, totalling over 70 papers on behavioural ecotoxicology in crustaceans to determine the main behavioural endpoints developed and any gaps in the current literature. Studies on behavioural effects are known to be fast and highly sensitive, with reported effects at concentrations up to 1000 fold lower than lethal concentrations. Despite this, standard ecotoxicology testing using acute lethality and chronic effects on growth, development, and reproduction have historically been used to define environmental quality standards. Behavioural endpoints have received far less attention largely due to a lack of the necessary tools to facilitate these studies. Recent computational advances have facilitated an increase in behavioural ecotoxicology due to a capacity to quantify a range of sensitive endpoints. This review has highlighted that most of the work in crustaceans has focused on swimming and avoidance behaviours (49% and 21% respectively). However, there are a large number of other quantifiable endpoints including reproduction, aggression, feeding, anti-predator and types of taxis. With closely related endpoints such as phototaxis and anxiety-like behaviours there is a need for clear definitions and carefully planned experiments for appropriate interpretation of effects. While there have been a wide variety of species used in these studies, endpoints were often species-specific and associated with the life history of the crustacean (e.g., mate guarding). Most studies have focused on traditional contaminants, such as heavy metals (37%), pesticides (27%) and PAH's (20%), with a recent increase in novel compounds. Behavioural assays have been used extensively in pharmacological testing. Despite being translated to environmental toxicology of fish, they are yet to be fully optimised to other taxa. With advances in the technology to facilitate behavioural studies in ecotoxicology, there is the potential for developing high-throughput techniques that measure multiple behaviours in crustaceans. However these are yet to be fully optimised and validated.

MO099

Behavioral and morphological responses of crucian carp (Carassius carassius) exposed to the antidepressant fluoxetine under predation risk

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Due to their potential for affecting the modulation of behavior, the effects of selective serotonin reuptake inhibitors (SSRIs) in the environment are particularly interesting with regard to interspecies interactions, and the non-consumptive effects induced by predators in prey organisms. Here, we tested the effects of the SSRI fluoxetine on fitness related behavioral and morphological traits of the crucian carp (*Carassius carassius*) in the presence or absence of a natural predator, the northern pike (*Esox lucius*). The crucian carp is known to change behavior (i.e. boldness and sociability) and morphology (body shape) in the presence of predators. Effects on crucian carp boldness, sociability, body weight and shape were recorded after short-term (two weeks) and long-term (three months) exposure to fluoxetine at 1 and 100 μg/L, and interactive effects with the added stress of predator presence were evaluated.

MO100
Sewage treatment works' effluent affects activity of Gammarus pulex (Amphipoda)
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The effluent of sewage treatment works (STW) is a complex mixture of domestic and industrial compounds, including pharmaceuticals, which have the capacity to disrupt the normal functioning of aquatic organisms. For the first time, the impact of these effluents on the activity of a freshwater amphipod has been quantified. *Gammarus pulex* were exposed to the effluents of two similar STW at levels of 50%, 100% and controls for 7 days. Measurements of distance travelled (mm) and velocity (mm s^{-1}) of individual shrimp were taken with the Noldus Danio Vision Behaviour system after 0h, 2h, 24h and 7d intervals. Readings were taken every 0.016s for 4 minutes with alternating sequences of 60 seconds light:dark. There was no significant difference found between the velocity nor distance between any test groups. However, there was significant differences seen in the 'acceleration' and 'deceleration' responses of *Gammarus*: individuals exposed to both effluents moved further and faster in the first 5 seconds the lights were switched on (P< 0.01). Typically, *Gammarus* maintained in 100% effluent increased (in both velocity and distance travelled) by ~40% compared to controls after 7 days. Significant differences between treatment groups developed after 24h exposure, were more pronounced after 7 days, and the effect was greater with increasing concentrations of effluent. Similarly, the activity of effluent-exposed animals did not diminish after the lights were switched off. For example, in the first 5 seconds of darkness animals in the 100% exposure group had an average velocity of 13.2mm s^{-1} compared to 5.3 mm s^{-1} in the control group. The increase was significant in one effluent (P< 0.01) but was not seen in the other effluent. There have been other reports of pharmaceuticals at environmentally relevant concentrations affecting the activity of amphipods. This study suggests that STW effluents can have a similar impact. As a negatively phototaxic species, any influence on the response of *Gammarus* to light could have wider ecological repercussions. Ongoing studies are investigating the behavioural response to specific pharmaceuticals.

MO101
Temporal profiles of behavioral alterations in zebrafish embryos and larvae exposed to pharmaceuticals
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The occurrence of neuroactive chemicals in aquatic environments is of growing concern due to the risks posed to humans and other animals. Aquatic organisms are particularly vulnerable due to their continuous exposure to variable mixtures of pollutants. In fact, recent studies demonstrated that environmentally relevant concentrations of neuroactive pharmaceuticals can affect population-relevant fish behavior such as aggression, feeding and reproduction. There is a need to develop and improve bioassays that can support the evaluation of neuroactive environmental contaminants on fish behavior. In this study, we investigated the temporal profiles of behavioral effects in zebrafish *Danio rerio* early life stages after static exposure to neuroactive pharmaceuticals. Zebrafish embryos and larvae up to 5 days post fertilization (dpf) were exposed to venlafaxine (serotonin norepinephrine reuptake inhibitor antidepressant), carbamazepine (voltage-gated sodium channel inhibitor and GABA receptor agonist anticonvulsant) and oxazepam (benzodiazepine derivative anxiolytic) at concentrations in the μg/L range (1 nM -10 μM). The selected compounds are relevant aquatic contaminants, and concentration ranges are representative of environmental situations. Embryos and larvae were exposed for short (during 24 h) and prolonged (starting at day 0) exposure periods, simulating acute and chronic exposure scenarios. Behavior was assessed at embryonic (2 to 3 dpf) and larval (5 dpf) stages. The following behavioral endpoints were measured: photomotor and startle responses (both embryos and larvae), and phototaxis and thigmotaxis reactions (larvae only). Exposure concentrations were measured at the beginning and at the end of exposure by LC-MS/MS. Results of this study are discussed regarding their ecotoxicological implications and environmental relevance. In addition, the methodological aspects for the application of behavioral assays in future ecotoxicological studies are considered.

MO102
EFFECTS OF NATURAL DYE ERYTHROSTOMINONE AND SYNTHETIC BASIC RED 51 ON ZEBRAFISH EMBRYOS DEVELOPMENT AND LARVAE BEHAVIOR
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Organic compounds present in natural sources have been used in several experimental conditions aimed to develop safe human health dyes as alternative to replace synthetic dyes, since these are precursors of mutagenic compounds. Nonetheless, in addition to a safe human health dye, an eco-friendly dye is desirable to be used by industry, once the international scientific community is concerned about environmental health protection. Thus, toxicity tests to non-target organisms have been used to evaluate adverse effects of dyes to the ecosystem. Zebrafish embryos (*Danio rerio*) are becoming an important alternative model in ecotoxicology due to its easy maintenance and breeding, likewise a well characterized early development and transparent embryos, which allow us to easily analyze survival, morphological alterations, and behavior. This study aimed to assess and compare the effects of the natural dye erythrostominone (ERY – extracted from microorganisms), its photodegraded product (DERY), and the synthetic dye Basic Red 51 (BR51 – used in cosmetic industry) on development of embryos and behavior of larvae of zebrafish. Zebrafish embryos were exposed to different concentrations of ERY, DERY and BR51. Hatching success, coagulation, edemas, malformation and mortality of embryos were recorded daily until 96 h post fertilization (hpf) in a stereomicroscope (SMZ800, Nikon) with coupled camera (Nikon LV-TV, Japan). Behavior was assessed by measuring the locomotor activity (total swimming distance, time, activity and velocity) of zebrafish larvae at 144 hpf exposure to sub-lethal concentrations of ERY, DERY and BR51, using ZebraBox and ZebraLab software (ViewPoint Life Science, Lyon, France). All tests were performed under controlled temperature (26ºC ± 1) and dark incubation in order to avoid degradation of ERY and BR51 or further degradation of DERY. Our results showed that both ERY and BR51 affected yolk sac morphology of embryos of zebrafish with LOEC of 7.5 mg/L. ERY also induced the formation of edemas on embryos of zebrafish exposed to concentrations above 7.5 mg/L. Moreover, behavior was impaired on larvae of zebrafish either exposed to ERY or BR51. Interestingly, DERY did not affected the parameters tested with embryos and larvae of zebrafish, suggesting that degradation of ERY is sufficient to prevent toxic effects induced by ERY. Thus, ERY could be potentially used as an alternative dye.

MO103
Chemosensory responses to thyroid hormone and thyroid hormone disruptors in North American bullfrog (Lithobates catesbeianus) tadpoles
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Endocrine disrupting compounds (EDCs) enter wastewater through anthropogenic sources and are not fully removed by wastewater treatment processes. As a result, EDCs are persistent in wastewater effluent at low concentrations and can still be biologically active. Consequently, EDCs are able to disrupt the endocrine system, which regulates growth and development in vertebrates. Some EDCs are thyroid active, and therefore can have agonistic or antagonistic effects on the thyroid system. The normal functioning of the thyroid hormone (TH) is important in vertebrates during early life stages for the development of many organs and tissues. The present study investigates the effects of THs and EDCs on chemosensory acuity in North American bullfrog (***Lithobates catesbeianus***) tadpoles. Amphibians are good sentinels for studying the effects of TH disruption because metamorphosis is almost entirely dependent on THs. Furthermore THs drive the development and remodelling of the entire olfactory system in tadpoles during metamorphosis. As a result, TH disruption has the potential to affect chemosensory acuity in tadpoles after exposure to EDCs. In this study premetamorphic tadpoles were exposed to environmentally relevant concentrations of thyroxine (T4), triiodothyronine (T3), and 17β-estradiol (E2) in the presence and absence of T3 and T4, as well as a cocktail of known EDCs. After exposures, behavioural experiments were used to detect changes in chemosensory acuity using I-maze behavioural arenas. A mixture of amino acids our lab has shown to elicit an avoidance response in bullfrog tadpoles was administered into the I-maze and the tadpole's response to the chemosensory cue was measured by tracking its position in the I-maze. Changes in chemosensory acuity and behavioural responses to TH disruption can serve as a strong link between TH disrupting chemicals and adverse ecological effects. In an aquatic environment chemical signals provide organisms with important information about nearby predators, mates, or food. Therefore, a change in chemosensory acuity can lead to adverse ecological effects. Studying changes in chemosensory acuity will provide a greater understanding of the ecological significance of EDCs present in wastewater and the implications their presence may have on amphibian populations in receiving waters.

MO104
Behavioral ecotoxicology modeling of freshwater clam valve rhythm in response to waterborne copper
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Various chemical analysis methods are labor-intensive, costly, and are not suitable

for application to the continuous and real-time *in situ* monitoring of water quality. The Asian clam, *Corbicula fluminea*, a filter-feeding bivalve living in the upper layers of sediments, is an economically important species with a wide distribution throughout the world. Because of their wide distribution, ease of collection, environmental sensitivity, and ability to accumulate high concentrations of heavy metals, *C. fluminea* are recommended by a number of studies as ideal species for biomonitoring purposes. The aim of this study was to determine the dose-response relationship of *C. fluminea* exposed to waterborne copper (Cu), which would enhance one's ability to design biomonitoring systems for continuous, *in situ* monitoring of Cu levels in water. Both valve closing and siphon extension were chosen as the behavioral endpoints in the present study. The ecotoxicological parameters of these two sub-lethal effects were estimated and compared to determine the sensitivity of both behavioral responses under Cu stress, a crucial metric when assessing the feasibility of such systems for biomonitoring within aquatic ecosystems. The improved valvometry technique allows tested bivalves with a free-range spontaneous situation to avoid stresses from experimental artefacts. We linked proposed valve daily rhythmic models and toxicodynamics-based Hill model to predict valve dynamic responses under different Cu exposures with a circadian valve rhythmic endpoint. We found Cu detection thresholds of 5.6 (95% CI: 2.1 – 9.3) and 19.5 (14.6 – 24.3) ug L^{-1} for response times of 300 and 30 min, respectively. We showed that when exposure Cu concentrations exceed 50 ug L^{-1}, the differential degree of valve rhythmic response has a noticeable sensing ability within 30 min. Our study implicates an early warning dynamic biomonitoring system that provides potential *in situ* detection of waterborne Cu by circadian valve activities in *C. fluminea*. We conclude that the proposed daily valve-rhythm model can be utilized to predict valve dynamic responses under different Cu-exposure concentrations, taking into account circadian valve-rhythm endpoints, while using a precise valvometry system to validate the simulation.

MO105
Why are mammalian focal species unaffected by chlorpyrifos applications? Integrating data on foraging-behaviour, exposure, and toxicokinetics
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Tier 1 wild mammal risk assessment indicates high risk from use of chlorpyrifos (CP) due to its high toxicity to mammals when tested in standard laboratory studies. However, when aiming to conduct a realistic risk assessment, it is important to understand the status and ecology of the mammalian community present in the target crop field. In this respect it is important to estimate basic population parameters of species breeding within the treated fields (long term survival and reproductive performance), their foraging behaviour and feeding ecology, and the spatial & temporal movement of the focal species to evaluate factors (both natural and anthropogenic) which may influence reproductive performance. In addition it is equally important to collect exposure and mechanistic data to support and help explain findings in the field. Several field studies have been conducted towards this aim. To gain information on the mammalian communities within the CP-treated fields a variety of methods were used depending on the goal of the study and the species in focus, which allows measurement and ranking of any factors influencing the local mammalian community, including the application of CP itself. The work undertaken over consecutive years gave a good insight into exposure of mammals utilizing treated fields/fruit orchards. In order to support field findings and provide a higher tier risk assessment for mammals, mechanistic information were also provided based on a body burden model describing Toxicokinetics (TK) and Toxicodynamics (TD). This presentation will describe the comprehensive and holistic approach which was followed, the new approaches for mammalian studies in risk assessment over consecutive years of data collection, and will explain why the high risk of mortality predicted by the Tier 1 risk assessment is not seen in the field.

Cost effective and ecological relevant approaches in environmental toxicology using invertebrate species (P)

MO106
Disruption of hypopharyngeal gland function in honey bees exposed to a dietary neonicotinoid (imidacloprid)
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The neonicotinoid imidacloprid impairs development of the honey bee (*Apis mellifera*) hypopharyngeal gland (HPG), but whether HPG function is correspondingly disrupted has been untested, which undermines the ecological relevance of HPG-related assays in pesticide risk assessment. To determine whether imidacloprid indeed disrupts HPG function, we therefore implemented laboratory and field studies, using enzymatic, molecular and behavioural endpoints. Specifically, we investigated whether dietary exposure affected temporal polyethism, using Radio Frequency Identification (RFID) technology to track hive traffic in free-flying bees. In the laboratory, we investigated whether dietary exposure affected the enzyme systems that support larval nutrition (major royal jelly proteins, or MRJPs) and social immunity (glucose oxidase, or GOX). Overall, we demonstrate that dietary exposure of honey bees to neonicotinoid pesticide is potentially capable of causing generalised disruption of HPG function, which implies that imidacloprid-induced changes in HPG development can lead to detrimental effects on colony function. We believe this provides some overall support for the ecological relevance of HPG-related assays in pesticide risk assessment involving sublethal effects on honey bees. It is evident that further work is needed to test the field-relevance of some of our findings (namely imidacloprid-induced disruption to nutrition and social immunity), as in some cases (temporal polyethism) transcriptional responses that we observed did not translate into functionally significant impacts on bee health, highlighting the need to cross reference gene expression bioassays with ecologically relevant behaviour. Our work highlights the potential utility of new enzymatic, molecular and behavioural ecotoxicological endpoints for risk assessment of sublethal effects on honey bees, if further work can clarify their ecological relevance to colony-level impacts on bee health under more field realistic exposures.

MO107
Deciphering mechanisms of malathion toxicity under pulse exposure of the freshwater cladoceran Daphnia magna
L.N. Trac, Danish Technical University; O. Andersen, Roskilde University; A. Palmqvist, Roskilde University / Department of Environmental Social and Spatial Change
The organophosphate pesticide (OP) malathion is highly toxic to freshwater invertebrates including the cladoceran *Daphnia magna*, a widely used test organism in ecotoxicology. To assess whether toxic effects of malathion are primarily driven by exposure concentration or by exposure duration, *Daphnia magna* was pulse exposed to equivalent integrated doses (duration x concentration), i.e. 3h x 16 μg/L, 24h x 2 μg/L and 48h x 1 μg/L. Following recovery periods of 3h, 6h, 24h and 48h the toxicity of malathion on different biological levels in *Daphnia magna* was examined by analyzing the following endpoints: survival and immobilization; enzyme activities of Acetylcholinesterase (AChE), Carboxylesterase (CbE), and Glutathione S-transferase (GST); and Acetylcholinesterase gene expression at the transcriptional level measured by qPCR. The results showed no difference in survival among equivalent integrated doses. Adverse effects were driven by exposure concentration rather than duration. Specifically, short pulse exposure to high concentration of malathion (3h x 16 μg/L) resulted in more immobilized daphnids, lower AChE and CbE activities and higher transcript level of AChE gene compared to long pulse exposure to low concentration (48h x 1 μg/L). The expression of the AChE gene was up-regulated, indicating a compensatory mechanism to cope with inhibition of the enzyme. In addition, for this OP insecticide, the severity of the effects increased during the postexposure recovery period. The results of this study indicate that Environmental Risk Assessment based on the standard OECD immobilization test may underestimate OP risk in certain situations, and emphasize the relevance of employing a realistic exposure test scenario. The study has helped elucidate the response mechanism at the molecular level of the target enzyme for OP pesticides, which was still questionable in previous studies. More work on cytochrome P450 (CYP), CbE and GST regulation during and after exposure could further improve the understanding of molecular responses of these enzymes to toxicity of OPs in *Daphnia magna*.

MO108
Does long-term fungicide exposure affect the reproductive performance of leaf-shredders?
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Leaf-shredding amphipods play a critical role in the ecosystem function of leaf litter breakdown, a key process in detritus-based aquatic ecosystems. However, these organisms' fitness and functioning may be adversely influenced by exposures to fungicides, a group of pesticides targeting conserved sites of toxic action, while the effects on shredders' reproductive performance have not yet been assessed. To fill this knowledge gap, a semi-static 56-day partial life-cycle bioassay with *Hyalella azteca* (ten animals/replicate; *n*=30) was performed. Two field relevant levels of a fungicide mixture (at a low and a high sum concentration of 5 and 25 μg/L, respectively) were tested, whereby the single fungicide concentrations in the low sum concentration (1 μg/L each) corresponded approximately to the fungicides' regulatory acceptable concentrations (according to the European Union's environmental risk assessment; EU-ERA). Endpoints related to the energy processing (leaf consumption and feces production), the

growth, and the reproduction (amplexus pairs, number, and length of offspring) of the amphipods were assessed. While leaf consumption was unaffected, exposure to both fungicide treatments significantly reduced amphipods' feces production (~20%) compared to the control, which may be indicative for an increased food utilization to compensate for stress-related energy demands. However, this mechanism did not fully compensate for the higher energy demand and might have caused trade-offs in the energy allocation among physiological processes: while growth remained unaffected, amphipods forming amplexus were less abundant in both fungicide treatments (only significant in the high treatment). As a result, time to release of first offspring was delayed in both treatments and the number of offspring was significantly lower in the high treatment, whereas offspring length was unaffected. Moreover, a 5-10% increase in mortality was observed in both fungicide treatments compared to the control, which may suggest a reduced allocation of energy to maintenance. The results of this study indicate that chronic fungicide exposures can negatively impact shredders' reproductive performance. This may translate into lower abundances and thus a reduced contribution to leaf litter breakdown, which may have detrimental consequences for detritus-based food webs. Moreover, this study provides further proof that the EU-ERA procedures for fungicides may not safeguard aquatic ecosystems.

MO109

Using in-situ bioassays with the aquatic snail Physa acuta to assess impacts of wildfires on water quality

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Wildfire is a common phenomenon in South-Europe, namely in Mediterranean countries as Portugal, where over the last decade an average of 144 000 ha burnt per year. It is known that wildfires affect aquatic systems altering both physical and chemical proprieties through the increased bioavailability and transportation of pyrolytic substances such as polycyclic aromatic hydrocarbons (PAHs) and metals associated to ash/soil loads. Hence, river ecosystems, particularly its biota, require a closer examination regarding wildfire effects. Once *in situ* assays address more realistic environmental multi-stress situations, an *in situ* bioassay at a Portuguese recent burned area (Miranda do Corvo, Coimbra) was performed to test reproductive effects by using the aquatic snail *Physa acuta*. Reproductive parameters, such as the total number of eggs, egg masses per snail, average of eggs per egg masses and the average of eggs and masses per snail were the end points tested at the end of the 8 days of exposure. The assay was performed at the time of the first major rainfall events and comprise four distinct sites: one reference site located in Ceira river upstream the burnt area (RUS); and three other sites, two of them located within the burnt area in tributary streams (SDS and SUS) and one downstream the burnt area in the Ceira river (RDS). Test chambers were elaborated accordingly to the needs of the pulmonate snail, allowing floatage and luminosity entrance. All the endpoints assessed indicate that RUS (the reference site located upstream the burnt area) was the site were reproduction was less affected, while SDS (a stream within the burnt area) was the site with lower reproductive outputs. Hence, *Physa acuta* reproductive assay demonstrate to be an effective and reliable test to discriminate among aquatic systems impacted by wildfires. Further studies, considering the embryo development should be also assessed.

MO110

Effects and biotransformation of the preservative methylisothiazolinone in the amphipod Hyalella azteca

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The preservatives methylisothiazolinone (MI) together with the trademark ‘Kathon CG’, comprised of methylchloroisothiazolinone (MCI) and MI in a 3:1 ratio, have been investigated extensively the past decade owing to an increase in allergic contact dermatitis. The preservatives are present in many products ranging from cosmetics and household products to water based paints, cooling tower water, industrial applications and toys, posing a possible risk of exposure in many aspects of life. MCI/MI have been used extensively and effectively as a preservative since the 1980s with a broad spectrum of activity against fungi, yeasts and bacteria at low concentrations. Since the millennium, MI has been used as an individual preservative alongside the MCI/MI mixture and other biocides and to some extent as a replacement of MCI/MI. The reason may be that MCI/MI is on the list of dangerous substances (i.e., R43/H317) and has to be declared when present in concentrations >15 ppm in cosmetics. No such requirements were present for MI up until 2005 where the maximum concentration in cosmetics were set at 100 ppm, a 25-fold increase from when it was in combination with MCI in the 3:1 ratio having a maximum concentration of 3.75 ppm. However, in 2015 EU declared a limit of 15 ppm MI in rinse-off cosmetics and no safe limit was specified for leave on cosmetics. In industrial products there is also a self-classification system with a limit of 0.1 % (1000 ppm), but it is not regulated as the self-classification name implies. Taking all of these sources into consideration the concentration of these compounds in water bodies may be higher than previously assumed and therefore pose a possible risk to the freshwater invertebrates living there. *Hyalella azteca* is an example of a well-distributed freshwater amphipod that is a well-established model organism in ecotoxicology. This study aims to investigate the effect of MI on *H. azteca*, as well as to explore MI biotransformation by *H. azteca*. Effects are assessed as changes in mobility and survival, and cellular reactions to reactive oxygen species (i.e., catalase induction, superoxide dismutase activity and lipid peroxidation) after MI exposure. Biotransformation is investigated by CYP induction and GST activity as well as disappearance rate with and without *H. azteca* present. Results from this ongoing study will be presented and discussed in relation to the current regulations.

MO111

Evaluation and improvements of a mayfly, Neocloeon (Centroptilum) triangulifer (Ephemeroptera: Baetidae) toxicity test method.

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A recently published test method for *Neocloeon triangulifer* assessedthe survival and growth of larval mayflies exposed to several reference toxicants (NaCl, KCl, and $CuSO_4$). Results were not able to be replicated in subsequent experiments. To identify potential sources of variability, the authors measured 7-day growth and survivorship in NaCl, using: (a) 1- vs. 3- vs. 5-day-old larvae, (b) 1-day-old larvae hatched from freshly collected eggs (not stored at cold temperatures) vs. eggs held in long-term storage at 4°C, and (c) 1-day-old larvae from five different laboratory cultures (two U.S. EPA, one USGS, one University of Illinois and one from University of Guelph). Fifty percent lethal concentration (LC50, mg/L) and 25% inhibition concentration (IC25, μm) point estimates were used to quantify survival and growth, respectively. Statistically significant differences in NaCl LC50s were observed among different ages of mayflies at test initiation (LC50=616.56, 989.43, 1667.24), with older organisms being less sensitive. No significant differences in growth could be elucidated between different aged treatments. Survival and growth of freshly collected eggs (LC50=701.27, IC25=626.56) differed significantly from eggs stored at 4°C for either three (LC50=394.13, IC25=424.47) or five (LC50=424.18, IC25=361.28) months, though no significant differences were observed between stored cohorts. A comparison of freshly collected eggs from 4 of the 5 different laboratory cultures tested do far showed no significant differences in survival or growth. Additional results will also be presented on larval age, storage, and laboratory source comparisons with KCl and CuSO4, and comparisons of freshly collected eggs vs 2 month old stored eggs at 10°C. To ensure consistent and sensitive test results we recommend continuing the use of 1-day-old larvae when using this species.

MO112

Toxic effects of wildfires on aquatic systems: in situ bioassays

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Wildfires constitute a diffuse source of contamination of aquatic systems affecting water quality, through the production and transport of pyrolytic substances such as polycyclic aromatic hydrocarbons (PAHs) and metals associated to ash/soil loads. However, the effects of these compounds from recently burnt areas on the aquatic biota have been poorly studied. Hence, the objective of this study was to assess the ecotoxicological effects of wildfires in aquatic systems. A sampling area, recently burnt, was selected near Miranda do Corvo (Portugal). Five sites were selected: two in the main water course of the watershed - Ceira river, being one located upstream (RUS) and the other downstream (RDS) the burnt area ; two in tributary streams within the burnt area (SUS and SDS); and finally one in a stream at an unburned area (CS). During the first rainfall events after the wildfire, distinct groups of organisms, including the water flea *Daphnia magna*, the freshwater shrimp *Atyaephyra desmaresti* the freshwater clam *Corbicula fluminea* and the mosquitofish *Gambusia holbrooki* were exposed in situ, in all five sites, using test chambers. With exception of *D. magna*, all other tested organisms can be found in Ceira river. After an exposition period of four days, the mortality and post-exposure feeding inhibition were evaluated. Feeding depression after exposure time was selected as a sub-lethal endpoint because it is a quick, sensitive and ecologically relevant indicator of toxic stress. The results showed a very low mortality for all the species and sites, thus this lethal parameter was not sensitive to discern impacts among the assessed sites. Conversely, the sub-lethal post-exposure feeding inhibition endpoint, revealed a decrease of feeding rate, in streams within the burnt area (SUS and SDS), that seemed to be the most affected

places in the study area. The sites outside the burned area, both on river (RUS) and on the stream (CS), showed no adverse effects in this endpoint. Hence, the current results showed that *in situ* bioassays were a suitable tool to assess the risks of wildfire to aquatic species and that the post-fire runoff can sub-lethally impair the aquatic organisms in water bodies located within or downstream the burnt area.

MO113

Influence of surfactants in the bioaccumulation kinetics and metabolism of selected pharmaceuticals in benthic invertebrates

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Nowadays, it has been widely demonstrated that elimination of many pharmaceuticals during conventional wastewater treatment is incomplete. Therefore, despite at low concentrations, these bioactive compounds are being discharged in natural waters on a regular basis and are consequently considered as semi-persistent contaminants[a]. The structural and functional equilibrium of freshwater ecosystems could be compromised by their environmental presence. However, data on this regard is still insufficient for a full risk assessment, particularly on their direct or indirect effects in the first trophic levels. Surfactants, on the other hand, are used in much higher quantities and also released into sewage systems or directly in surface waters. Their environmental presence and potential ecotoxicity have been thoroughly studied[b]. Benthic invertebrates are key in the functioning of aquatic ecosystems, as they are involved in nutrient recycling and represent a relevant food source for other invertebrates and fish[c]. The amphipod *Gammarus fossarum* is frequently used as sentinel species of water quality, as it is widespread in western Europe and its physiology is well known. Recent works have shown the bioaccumulation of selected pharmaceuticals in gammarids[d,e], but have not fully addressed either their bioaccumulation kinetics or their capacity to metabolize the toxicant. The main goal of this work is to study the role of a mixture of frequently detected detergents in the bioaccumulation kinetics of selected pharmaceuticals in *G. fossarum*, evaluating their potential synergetic or antagonistic effects. Laboratory exposure experiments for both pharmaceuticals and detergents (concentration ratio 1/25) were set up for three individual compounds: the anxiolitic oxazepam, the anti-inflammatory ibuprofen and the antibiotic sulfamethoxazole. The feeding rate was selected as end-point of the experiment. Gammarids samples were processed using microQuEChers extraction and pharmaceuticals bioaccumulation and metabolism was followed-up by means of nanoliquid chromatography coupled to MS/MS (nanoLC-MS/MS) and high resolution mass spectrometry (nanoLC-HRMS), aiming to elucidate potential transformation products. Kümmerer, K. Springer Ed. 2008 Ying, G. et al. Environ. Int. 32(2006), 417-431 Besse, J.P. et al. Water Res. 47(2013) 650-660 Berlioz-Barbier, A. et al. J. Chrom. A 1367 (2014) 16-32 Miller, T. et al. Sci. Of the Total Environ. 511(2015) 153-160

MO114

Eliminating Stressors in Laboratory Tests with Chaoboridae and Corixidae

F. Joyce, S. Taylor, Cambridge Environmental Assessments

The sensitivity of organisms tested under laboratory conditions may differ significantly to those exposed to contaminants in their natural environment. Standard procedures for laboratory invertebrate bioassays operate under optimal laboratory conditions specified in OECD guidelines, which are generally ‘sterile’, e.g. void of natural refugia and food. However, when using feral organisms, the change from field to laboratory conditions can cause additional stress, thus potentially affecting the outcome of acute toxicity tests for use in regulatory risk assessments of plant protection products. The need for test modifications for some taxa based on individual species life-histories and habitat preferences are important to consider for successful testing of non-standard invertebrates. For example, the acclimatisation period and testing conditions in the laboratory can be modified depending on the requirements of different species in their natural environment and their response to stressors such as light levels and temperature. Here we will present examples taken from two invertebrate taxa (Chaoboridae and Corixidae) for which control mortality during acute toxicity tests performed by Cambridge Environmental Assessments was anomalously high in comparison to standard organisms. We will discuss the subsequent research and trials carried out to mitigate non-chemical stress and increase control survival and reliability, and also provide recommendations for the potential development of standard operating procedures for non-standard invertebrate work.

MO115

Culturing and Testing Early Life-History Stage Larvae of Chaoborus crystallinus and Cloeon dipterum

M. Allen, S. Taylor, A. Howells, F. Joyce, F. Radford, Cambridge Environmental Assessments

The juvenile stage of freshwater invertebrates are regarded as the most sensitive life-history stage to the toxic effects of exposure to Plant Protection Products (PPPs). Standard invertebrate testing approaches e.g. *Daphnia Magna*(OECD 202), Chironomid (OECD 235) use Here we will present the results of the development of testing approaches for two non-standard invertebrates, the mayfly (*Cloeon dipterum*) and the phantom midge (*Chaoborus crystallinus*) using specific life stages. Organisms were collected from outdoor mesocosms at Cambridge Environmental Assessments (CEA) and cultured in the laboratory. Mayfly and *Chaoborus* cultures were maintained in the laboratory within mesh cages so that adults would emerge and re-lay their offspring within the cultures. As this method was unsuccessful we attempted a range of alternatives to breed these species under controlled conditions. For example, beakers of de-ionised water were added to the *Chaoborus* culture cages and a period of low light at dawn and dusk was introduced to encourage oviposition for both species. This proved temporarily successful for *Chaoborus*, however continued breeding did not occur. Mayflies failed to breed under the different conditions. In order to standardise our higher tier tests, larval stages 1 and 2 for mayflies were identified and *Chaoborus* within the size range 5-10 mm were used within the acute toxicity test. Although the results of this work did not meet the objectives that we set out to achieve (i.e. organisms of a known age were not able to be used), this presentation will show our experiences and also recommend next steps for further refinement of culturing and testing methods.

MO116

Magnetic particles' effects on survival and hatching of the rotifer Brachionus calyciflorus and the growth of the Chlorella sp. alga.

M.. Salcedo, I. De Vicente Ãlvarez-Manzaneda, University of Granada / Ecology

The main objective of this research is to study the possible toxicological effects of iron magnetic microparticles on the rotifer *Brachionus calyciflorus* and the cloroficean alga *Chlorella* sp. These magnetic microparticles have recently been proposed for the restoration of eutrophic aquatic ecosystem, for remove the phosphorus located in them. With the aim of carrying out the toxicological test on *B. calyciflorus*, it was followed ASTM protocol. During 24 hours the organisms were in contact with different concentrations of magnetic particles, analyzing the mortality of the organisms. On the other hand, a test about the rotiferb_s eggs hatching in contact with dissolved iron from the particles was carried out. In the case of the test with the alga *Chlorella* sp., OECD’s standardized protocol was followed. This test last 72 hours and they were used different concentrations of magnetic particles, after these 72 hours the inhibition growth of the alga was calculated. The results obtained were a CE50 of 1.63 g/l of iron for the rotifers and 0.085 g/l for *Chlorella* sp., the alga was more sensitive to the particles than planktonic organism being 100% of algal growth inhibition from 0.5 g/l. Hatching of *Brachionus calyciflorus* eggs in contact with the dissolved iron was total.

MO117

Effects of Chernobyl-derived radionuclides on fluctuating asymmetry and fecundity in Asellus aquaticus: 30 years on

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The effects of long-term, environmentally relevant doses of radiation on biota remain unclear owing to a lack of studies following chronic exposure in contaminated environments. Such studies are necessary to elucidate and mitigate the impacts of both planned and accidental releases of anthropogenic radioactivity on the environment. This field study aimed to determine the effects of chronic exposure to environmental radioactivity on an aquatic isopod, *Asellus aquaticus* using two cost effective biomarkers, fluctuating asymmetry and fecundity. Fluctuating asymmetry is defined as random deviations from the expected perfect bilateral symmetry of an organism and has gained prominence as an indicator of developmental stability in both ecotoxicological and ecological studies. *Asellus aquaticus* is a benthic detritivore commonly used in ecotoxicity testing of sediment-borne contaminants. Specimens were collected from littoral zones of six lakes with varying degrees of contamination from the Chernobyl incident in Belarus and Ukraine. Estimates of external dose rates at sample sites ranged from 0.01 to 35.9 $\mu Gy/hr^{-1}$ Levels of asymmetry were assessed using five independent traits measured using ImageJ. Mean brood size, weight of broods and brood sizes normalised to maternal weight were used to assess reproductive output at sites. A two-way ANOVA revealed significant differences in pooled levels of mean asymmetry between sites. However, there was no correlation of asymmetry with estimated external doses suggesting that differences in asymmetry were not attributed to radionuclide contamination. Further, no significant differences in weight-normalised brood sizes and weights were exhibited between asellids collected from sites along the contamination gradient. All brood sizes and weights of Chernobyl asellids were within the range of reference sites. These results indicate that following 30 generations of radiation exposure, there are no detectable effects on developmental stability and reproduction in asellid populations. Studies are ongoing to determine the genetic variability and potential adaptation of these invertebrates to provide protection from ionising radiation. These will findings enhance our understanding of the response of biota to chronic pollutant exposure and will help elucidate long term effects of large scale nuclear incidents such as Chernobyl and Fukushima.

MO118

Culturing and Testing Early Life-History Stage Larvae of Asellus, Crangonyx, Lymnaea

F. Pickering, S. Taylor, A. Howells, F. Radford, Cambridge Environmental Assessments

The conduct of acute aquatic toxicity testing in the laboratory provides an efficient means of predicting the effects chemicals may have in the environment. Standard invertebrate testing approaches use age specific organisms e.g. Daphnia magna (OECD 202, 2004) and Chironomid (OECD 235, 2011) to eliminate potential differences in sensitivity at varying life-stages. However, there is an increasing need for the development of standard culturing and age recommendations for non-standard invertebrates used in higher-tier toxicity testing. We will present the results of recent work carried out by Cambridge Environmental Assessments (CEA) in this area for culturing age specific non-standard invertebrate species for use in acute laboratory studies. A range of freshwater species were selected for age specific culturing and testing namely: *Lymnaea stagnalis*, *Crangonyx pseudogracilis*, and *Asellus aquaticus*. Organisms were collected from outdoor mesocosms and bred in the laboratory and juveniles produced were isolated periodically to ensure age specific juveniles for testing. We present the methods used for collecting, acclimating and culturing these freshwater invertebrates and also make recommendations based on the suitability of organisms selected and recommend ages for use in testing. In addition, we will highlight the need for any further research and development required.

MO119

Rediscovering a simple and precise methodology to assess filtering rate quantification for bivalve: Neutral red dye

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Neutral red is a weakly cationic dye soluble in water, with a low toxicity for almost all class of organisms, for which has been used as histological and vital stain since early twentieth century. Estimating the amount of water filtered by bivalves is one of the earliest applications of neutral red. However we found less than twenty studies applying this methodology since its description in 1954. The aim of our study was to optimize the methodology of filtering rate based on the solution of neutral red by addressing the effect of i) acidifying pH; ii) holding temperature before spectrophotometric reading; and time iii) before and iv) after acidification of solutions of neutral red used for filtering assays. Furthermore, as a case study we fine-tuned the filtering assay for the estuarine bivalve, *Cerastoderma edule*, as potential species to be used for estuarine monitoring purposes. Results showed not statistically differences in absorbance of neutral red solutions holding at 4 or 20ºC, neither in solution acidified between the range of pH 4-5. However, absorbance at 545 nm decreased significantly as pH of solutions increase at pH 6. The time before acidification has not a significant effect on absorbance. However, once the neutral red solution is acidified, the absorbance of the solution tends to decrease over time for which is recommended read the absorbance in the first 24h. Finally, our results showed that the sensibility of this technique decrease at low concentrations, for which is recommended used neutral red concentration enough to allow, after the filtering period, final concentrations above 0.5 mg/L. For *C. edule* the filtering conditions per individual were optimized as 100 ml of 4 mg/L of neutral red dye during 30 min period in dark conditions. The Cd 48-h EC50 postexposure filtering for *C. edule* was 3.4 folder lower than the 48-h LC50 (0.33 vs 1.13 mg/L, respectively), confirming filtering rate as a more sensitive and cost-effective endpoint for this bivalve than mortality. The bioassay using the bivalve *C. edule* based on postexposure filtering rate based on this simple colorimetric technique appears to have potential as a tool for environmental estuarine assessment. Future development of field assays based on postexposure filtering rate (namely, in situ assays) with this species is promising to use in monitoring programmes. We expect that the new harmonized protocol became in a widely used cost-effective tool for monitoring filtering rates.

MO120

Earthworms (Eisenia fetida) response to short-term exposure to triclosan

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Triclosan (TCS) is a broad-spectrum antimicrobial and antifungal agent and it is a common constituent of household and personal care products. TCS widespread use has resulted in its introduction into environment and has raised concerns regarding possible adverse effects on terrestrial and aquatic organisms. Earthworms *Eisenia fetida* were exposed to 10-750 mg/kg of triclosan for 14 days. Mortality and biochemical responses including the activity of catalase (CAT), superoxide dismutase (SOD), glutathione reductase (GR) and malondialdehyde (MDA) were examined after 4, 7 and 14 days. None of the tested TCS concentrations evoke earthworm mortality during the experiment. Triclosan exposure reduced the growth of earthworms. Short time exposure (up to 14 days) showed, that highest activity of catalase was observed after 4 days exposure and by increasing TCS exposure time catalase activity was dereasing. Highest superoxide dismutase and glutathione reductase activity was detected after 7 days exposure of triclosan.

MO121

Passive dosing of hydrophobic organic chemicals in toxicity tests with Caenorhabditis elegans

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The standardized toxicity test with the nematode *Caenorhabditis elegans* (ISO 10872) is widely used for toxicity testing of chemicals. Chronic toxicity endpoints such as reproduction are thereby preferably assessed because of their higher sensitivity and ecological relevance compared to acute mortality. Due to its simple, cost-efficient cultivation and short generation time, *C. elegans* is particularly suitable for high-throughput toxicity testing. Testing sub-lethal parameters such as reproduction requires feeding with *Escherichia coli* cells during the test. However, the bacteria can act as sorptive sink and, therefore, reduce the freely dissolved, bioeffective concentration (C_{free}) of hydrophobic organic chemicals (HOCs). This results in poorly defined exposure conditions and reduced test sensitivity when actively spiking the HOCs into the test medium by using an organic solvent as solubilizing agent. Passive dosing is a promising technique for controlling and maintaining constant C_{free} in small-scaled toxicity tests since potential decreases of C_{free} are compensated by continuous partitioning of chemicals from a saturated reservoir. This study investigated the applicability of passive dosing of HOCs from silicone O-rings in the chronic *C. elegans* toxicity test. 10 polycyclic aromatic hydrocarbons (PAHs) that cover a broad range of hydrophobicity were tested as model chemicals. During all experiments, C_{free} of test chemicals was quantified by solid-phase microextraction (SPME), either in test medium or via headspace. Studies on release kinetics of test chemicals revealed that after preincubating the silicone O-ring in the test medium for 24 hours at 60 rounds per minute most PAHs reached equilibrium partitioning. During the 96 hours of *C. elegans* toxicity test, C_{free} of PAHs remained constant when applied by passive dosing, whereas C_{free} decreased by ≥ 90% until the end of the test when actively spiking the PAHs. This yielded in considerably lower EC_{50} values when exposing *C. elegans* by passive dosing compared to active spiking. As expected, neither C_{free} nor toxicity of PAHs decreased with increasing *E. coli* cell densities in test media when applying PAHs by passive dosing. In summary, passive dosing is a promising tool for assessing the chronic toxicity of HOCs with *C. elegans* whereby SPME proved to be suitable for verifying C_{free}.

MO122

A decade of progress on freshwater mussel toxicity testing and opportunities for further advancement

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Freshwater mussels belonging to the family Unionidae include more than 670 species of bivalves worldwide. Many of these taxa are imperiled globally and IUCN lists 28 as extinct and 106 as endangered or critically endangered. Effects of toxicants are reasonable hypotheses to test as limiting factors because aspects of mussel life history make them vulnerable to degraded water or sediment quality. Juvenile and adult mussels are benthic suspension and deposit feeders exposed to pollutants in surface water, sediment, and pore water and through ingestion of filtered particles with sorbed contaminants. Mussel toxicology has advanced since the 2006 ASTM International *Standard Guide for Conducting Laboratory Toxicity Tests with Freshwater Mussels (E1706)* provided a foundation for evaluating the quality of toxicity tests conducted with early life stages of mussels to dozens of chemicals. Mussels are known to be sensitive (relative to other freshwater organisms) to ammonia, some metals and ions including copper, nickel, lead, zinc, chloride, sulfate, and potassium. However, the science lacks full identification of pollutants that may limit survival, growth, reproduction, behavior, recruitment and recovery of mussels because few of the potential causative chemicals have been evaluated in the laboratory. Also, toxicity tests seldom address mussel reproduction or recruitment with test durations short (e.g., typically 1- to 28-d) relative to mussel lifespans (e.g., typically 10- to 60-y). Advances in mussel feeding and husbandry have promise for conducting longer-term exposures evaluating growth and behavior. Developing standard methods for sediment toxicity testing and effluent toxicity testing with mussels may advance the field, as the water-only ASTM guideline did a decade ago. The vast majority of mussel species remain untested, and interspecies variability in chemical sensitivity has been examined and modeled, but not explained through mechanistic and or genomics approaches for informed species-selection in toxicity testing or risk assessments of untested species. To aid habitat evaluation, studies are needed to better characterize exposure (including sediment, pore water, and food influences) and to provide benchmarks to define acceptable pollutant concentrations in water, sediment, and diets. Field confirmation of benchmarks predicted by laboratory toxicity tests is important in determining the significance of pollutants and in design of ameliorative measures.

MO123

Comparing Single species Toxicity Tests to Mesocosm Community-Level Responses to Total Dissolved Solids Comprised of Different Major Ions.

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Total Dissolved Solids (TDS) dosing studies representing different sources of ions were conducted from 2011-2015. Emergence responses in stream mesocosms were compared to single-species exposures using a whole effluent testing (WET) format and an *ex-situ* method (single species tests in containers receiving mesocosm water). The first 4 years used a dose-response design for each year's TDS recipe, which differed based on the relative dominance of major ions. The 2015 experiment tested three of the previous year's recipes dosed simultaneously but at only one level of moderate specific conductivity (ca. 850 uS/cm). A mayfly, *Neocloeon triangulifer,* was incorporated for testing alongside the standard WET test organisms. Overall, *N. triangulifer* tended to be more sensitive than other standard test organisms, but there were differences in sensitivity between the *ex-situ* method and WET tests. *N. triangulifer's* sensitivity in the *ex-situ* exposure reflected that of mayfly emergence from the mesocosms. Caddisfly emergence was generally as sensitive to TDS exposures as mayflies in 2011-2014 tests, although less sensitive when the excess TDS was dominated by Cl^- salts. However, caddisfly emergence was not significantly different from the controls and therefore, not as sensitive as mayfly emergence for the 2015 constant conductivity study. Chironomids were less sensitive to excess TDS than Ephemeroptera and Trichoptera taxa when TDS was dominated by Cl^- and $NaHCO_3$, but appeared more sensitive when the TDS was a mixture of SO_4^{2-} and HCO_3^-. Mayfly emergence was the only emergence endpoint that demonstrated a clear and consistent adverse response to excess TDS. Emergence in the control was always the highest and sulfate the lowest. Cl^- and HCO_3^- were intermediate and similar in response, but overall significantly lower than the control. In the WET format, mayfly growth in bench tests was: control > Cl^- = SO_4 > HCO_3^-. For the 2015 experiment, where Cl^-, HCO_3^-, and SO_4 were adjusted to establish a conductivity of approximately 850 *u*S/cm, the 20% hazardous concentration (HC20) indicated that deleterious effects to mayflies occurred at this conductivity regardless of ion composition. These results suggest that stream benthic communities are significantly more sensitive to excess TDS from coal mining activities that leave leachable spoils rich in carbonate and sulfate than to produced waters emanating from deep oil and gas wells that tend to be dominated by sodium and calcium chloride.

MO124

Comparative toxicity assessment of lead on two Daphnid species

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Water is an essential resource for humans, economy and ecosystems and often can be under risk of contamination. Chemical contamination of anthropogenic source is generally related to industry, agriculture and urban activities. Lead is a naturally occurring metal, but high levels can be found in the environment due to antropogenic actions. Thereby, this study aimed to compare the toxicity of lead to two different Daphnid species, *Daphnia magna* and *Daphnia similis*, which are species from different climate scenarios. To accomplish this goal three toxicity tests were performed with both species. To predict the letal concentration (LC) of lead to these organisms an acute immobilisation test (OECD 201) was carried out. Regarding sublethal effects, Pb effects on the reproductive output and female growth was achieved through the reproduction tests (OECD 211). In addition, and looking at one of the functional traits of daphnids as filter feeders, feeding inhibition tests were carried out based on the protocol developed by McWilliam & Baird (2002). Comparing lethal concentrations for *D. magna* and *D. similis* it is noteworthy that the $LC_{50\text{-}48h}$ values were similar, with *D. magna* showing a slightly higher value ($LC_{50\text{-}48h} \cong 0.45$mg/l) than *D. similis* ($LC_{50\text{-}48h} \cong 0.36$mg/l). Regarding the chronic assay, *D. magna* was more influenced by Pb on the reproductive output and *D. similis* on growth. A similar pattern was also attained in the feeding inhibition tests. According to the obtained results we can see that Pb affects both daphinids in different ways, as also shown for other chemical compounds.

MO125

The application of the Weight of Evidence Approach as an integrative tool for ecotoxicological risk assessment in Iberian rivers

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Chemical pollution is one of the most important threats to aquatic systems. The diversity of compounds present in these systems and the dynamics and interaction of these compounds with physical (water, sediment) and biological (amphibian, fish, invertebrate, algae) compartments make difficult to analyze the effects and to give solutions for appropriate management. European directives propose the use of different quality indicators to evaluate the environmental status of aquatic ecosystems and recommend combining chemical and biological analyses to determine the final quality status and facilitate interpretation of the results. Therefore, we adopted a "Weight of Evidence" approach (WoE) to perform a toxicological risk assessment of four threatened rivers of the Iberian Peninsula: the Ebro, the Llobregat, the Júcar and the Guadalquivir. The study comprised chemical and ecotoxicological information, gathered within the context of the SCARCE project. The lines of evidence (LoEs) included in this study were: ? Chemistry: included chemical data (emerging contaminants and priority substances –PS–) from both sediment and water compartments ? Toxicology: included three different sub-lethal toxicity tests (*Physella acuta* in situ reproduction test, *Daphnia magna* in situ feeding test, *Chironomus riparius* sediment exposure test) and also sediment Toxic Units (TUs) for *Daphnia magna* ? Ecology: included invertebrate benthic community richness and percentage of Tr. Chironomini and Oligochaeta (considered pollution-tolerant species) The general picture obtained from this risk assessment showed that the invertebrate communities of the four studied rivers were seriously affected by chemical pollution. Of the 20 sites studied, 17 presented a bad ecotoxicological status (Ecology and Toxicology LoEs) and only 2 sites of the Júcar presented a good ecotoxicological status. Among the PSs, we identified PFOS, organophosphorus pesticides (mainly chlorpyrifos), alkylphenols (mainly nonylphenol) and different metals (e.g. Ni, Pb) as the main specific drivers of the risk in most of the sites. The chemical and ecotoxicological LoEs gave complementary results, providing evidence on the need to include a set of different criteria to assess causality for a correct ecotoxicological risk assessment.

MO126

Effects of Copper-Chromium-Borate wood preservative at different level of biological organization of the earthworm E. andrei

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Earthworms are commonly used organisms in ecotoxicology to assess soil quality or effect of chemicals on the soil fauna. In this study, the effects of a biocide, used for outdoor wood preservation and containing a mix of chromium, copper and borate (CuCrB), were evaluated at different levels of biological organization using the earthworm *Eisenia andrei*. Reproduction and behavioral endpoints were assessed using standardized ecotoxicological tests. Additionally and considering the important role earthworms play in organic matter breakdown, functional impacts were evaluated by measuring the feeding activity of the earthworms exposed to the biocide. For this purpose, the usefulness and feasibility of using the bait lamina method, which measure the disappearance of a series of organic bait ("bait") embedded in small PVC sticks inserted vertically into the soil, was investigated in a short (48h) and simple test under laboratory condition. Finally, the impact of the biocide was assessed at the molecular level, looking at metallothionein (mt), phytochelatin synthase (pcs) and cytochrome c oxydase 3 (cox3) gene expression after 48 hours and 28 days of exposure. Exposure to the CuCrB wood preservative affected the earthworm feeding activity and behavior to a greater extent than reproduction. An induction of the pcs gene was observed after 48h whereas is expression was stable after 28 days of exposure. The mt gene expression however was stable after 48h but was induced after 28 days of exposure. The respiratory chain seems to be affected in the presence of the biocide as the cox3 gene was repressed in exposed earthworms. These combined approaches allowed to assess the effects of pollutants from molecular to functional levels, bringing to light different impacted functions in the CuCrB exposed earthworms. Moreover, the use of the bait lamina test to assess the feeding activity of earthworms is a relatively simple laboratory method that allows the rapid screening of soil samples for sublethal effects due to the impact of chemicals, and also an assessment of the functional aspect of soils under standardized conditions. This approach shows promise for the a priori ecological risk assessment of chemicals.

MO127

Planarians in ecotoxicology: Effects of phenanthrene on Dugesia tigrina

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Freshwater planarians are animal models for developmental and regeneration research and are being rediscovered as useful animals in neuropharmacology and

ecotoxicology. Such interest is based on their unique characteristics, such as a stem cell population that originates all other cell types and provides them with the ability to regenerate complete body parts including their nervous system which has similarities to that of vertebrates. Furthermore, a plethora of endpoints can be easily measured and quantified in these flatworms, including behavioral, regeneration, reproduction and biochemical parameters. Therefore, planarians have the potential to provide new insights on the effects of some well-studied and ubiquitous compounds, such as polycyclic aromatic hydrocarbons (PAHs). The sexual planarian *Dugesia tigrina,* a common freshwater predator, was used to study the effects of phenanthrene. The estimated 96-hour LC_{50} for phenanthrene was 830.62 µg/L, while acute EC_{50} for head loss was 483.06 µg/L. Sub-lethal endpoints such as locomotion, feeding and regeneration were measured over 8-day chronic exposures. After 8 days, behavior of animals exposed to the highest phenanthrene concentrations was affected, with exposed animals travelling smaller distances and showing toxic anorexia in comparison to controls. This study provides new data on the toxicity of phenanthrene to invertebrates, showing that exposure to this PAH can result in adverse effects to *D. tigrina* well below the concentration needed to induce mortality.

MO128

The effects of DEHP, an endocrine disrupting chemical (EDC), on the Egyptian cotton leafworm, Spodoptera littoralis

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Di-(2-ethylhexyl)-phthalate (DEHP)[1] is a plasticizer widely used in plastics to increase flexibility and known to have endocrine disrupting effects on vertebrate species. Endocrine disrupting chemicals (EDC) are pollutants which can alter the health of an organism, its progeny or even a (sub)population, by interacting with the hormonal system. In the frame of the ANR DISCO project (endocrine Disruption of Insect Sexual COmmunication), the current project focuses on the effects of DEHP on a crop pest: the Egyptian cotton leafworm. As EDCs are known to have major effects during early development and the development of reproductive tracts, we first investigated the effects of DEHP on the post-embryonic development (length and number of larval instars, larval weight and food consumption) and the sex ratio of *S. littoralis*. To study the effect of DEHP at population scale, we then focused on its effect on the female sex pheromone detection by males. Indeed, this crucial process for mating is under endocrine control in our species[2] and could be potentially disrupted by DEHP. Besides, we investigated the potential modifications in the ecdysteroid titration in the hemolymph of larvae and adult males, using Enzyme Immuno Assay (EIA)[3]. For both those experiments, we chose to feed larvae, from the end of the 2nd larval instar to the last larval instar with either food with ethanol (control) or contaminated food at several concentrations (from 10pg to 40mg DEHP per gram of food). Most of the experiments are still in progress. Preliminary results showed that DEHP is weakly toxic for *S. littoralis*. Mortality is increased only for the two highest concentrations. DEHP would reduce the percentage of males at 1ng DEHP per gram of food and some effects on larval growth rate were recorded and have to be confirmed in additional experiments. [1] European Chemical Agency – ECHA (2008). European Union Risk Assessment Report – bis(2-ethylhexyl) phthalate (DEHP). JRC45705. ISSN: 1018-5593. [2] Bigot Laetitia, Abdul Shaik Haq, Bozzolan Françoise, Party Virginie, Lucas Philippe, Debernard Stéphane, Siaussat David (2012). Peripheral regulation by ecdysteroids of olfactory responsiveness in male Egyptian cotton leaf worms, *Spodoptora littoralis*. Insect Biochemistry and Molecular Biology. 42:22-31. [3] Porcheron, P., Morinière, M., Grassi., J., Pradelles, P. (1989). Development of an enzyme immunoassay for ecdysteroids using acetylcholinesterase as label. Insect Biochemistry. 19:117-122.

MO129

The effects of sublethal doses of pollutants on crop pest, Spodoptera littoralis

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Pesticides have long been used as the main solution to limit agricultural pests but their widespread use resulted in chronic or diffuse environmental pollutions, development of insect resistances and biodiversity reduction. The effects of low residual doses of these chemical products on organisms that affect both targeted species (crop pests) but also beneficial insects became a major concern, particularly because low doses of pesticides can induce various effects. In addition to the negative effects, some studies highlighted unexpected positive - also called hormetic - effects on insects, leading to surges in pest population growth at greater rate than what would have been observed without pesticide application. The present study aimed to examine the effects of sublethal doses of various representative products of large pesticide families used against a major pest insect, the cotton leafworm Spodoptera littoralis, and known to present a residual activity and persistence in the environment. Using an integrated approach from genes to behavior, we studied the impact on the peripheral olfactory system and the sexual or feeding behavior of our crop pest model following application of sublethal doses of deltamethrin, methomyl and chlorpyrifos. Whereas sublethal doses of methomyl appeared to disrupt the feeding behavior of larvae, we demonstrated a hormetic response of males to sublethal dose of deltamethrin. We completed our study by molecular (qPCR), biochemical (proteomic, AChE activity and metabolic) and electrophysiological approaches in order to decrypt the involved mechanism in pesticide response as well as in the behavioral disruption.

Fate, Effects and Risk Assessment of Chemicals in Aquatic and Terrestrial plants (P)

MO130

A novel flow-through system adapted from OECD 201 test to assess algae toxicity for difficult substances

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The OCDE 201 algal test is historically performed in static conditions where media are not renewed during the 72h exposure. Even if this method is sufficient for chemicals which are stable in water, this is often not the case for substances which degrade either abiotically (due to hydrolysis or volatilisation) or biotically (due to biodegradation or algal metabolism). For such substances, toxicity to algae is more difficult to determine due to probable difficulties in maintaining substance concentrations throughout the test. In order to generate high quality toxicity results where the substance was present throughout the test, Rovaltain Research Company has developed an innovative dynamic test system, where algae are exposed to a constant concentration of chemical over the entire test period. This work has been carried out as a part of the DAMIER project, a French funded project for the development and the use of High Accuracy QSAR models for REACH compliance. Indeed high quality data provided by this new method have been used to validate toxicity predictions of High Accuracy QSARs (HA-QSARs). After having designed and developed the experimental device (an "algaestat"), preliminary tests using a range of chemical substances known for their instability (volatility, photosensitivity,…) provided encouraging results. These preliminary results confirm the relevance of such flow-through systems to obtain high quality toxicity data for substances which are less stable under OECD 201 test conditions.

MO131

An efficient bioassay using Chlorococcum infusionum in soil media

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As a food source for terrestrial organisms, soil algae can be alternatively used for assessing soil toxicity. However, there was a limitation to collect soil algae from the soils during soil bioassay. In this study, we performed paper-disc soil method for evaluating soil algal bioassay. Growth zone, biomass, and photosynthetic activity of representative soil alga *Chlorococcum infusionum* were analyzed in the nickel-contaminated LUFA 2.2 soil. As a result, we observed the reduction of growth zone and biomass, and inhibited photosynthetic activity after 6d-exposure. The result of this study could be indirect soil quality indicator in terms of user-friendly and efficient assay for screening soil toxicity. *This subject is supported by Korea Ministry of Environment as the GAIA project (2014000560001) and by the Basic Science Research Program of the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT and Future Planning (2014R1A2A1A11050513) and the Ministry of Education (2013R1A1A2061386).*

MO132

Aquatic Macrophyte Species Sensitivity Distribution (SSD) - Selection of Potential Testing Species

G. Gonsior,

Due to a decision from the EFSA (European Food Safety Authority) further aquatic plant species should be tested for the risk assessment of growth regulators and herbicides. Therefore a broad range of additional species were selected and tested (*Heteranthera zosterifolia, Lysimachia nummularia, Egeria densa, Vallisneria spiralis, Callitriche palustris, Ranunculus aquatilis, Hottonia palustris, Mentha aquatic, Myriophyllum sibiricum, Nymphoides peltata, Veronica beccabunga, Potamogeton natans, Utricularia vulgaris, Ceratophyllum demersum, Glyceria maxima, Cabomba caroliniana, Wolffia arrhiza, Spirodela polyrhiza, Lemna trisulca*). The studies were performed based on OECD 239: Water-sediment *Myriophyllum spicatum* Toxicity Test or on OECD 221 Lemna sp. Growth Inhibition Test. Data will be presented with the focus on the comparability to the existing guidelines.

MO133

OECD 239: Influence of pH on the Growth of Myriophyllum spicatum

G. Gonsior,

Some herbicides show a fast degradation in test medium triggered by pH. *Myriophyllum spicatum* is able to influence the pH in a significant way during testing. Therefore, it might be advisable to stabilize test items for example in a more acidic water-sediment system to provide a refined risk assessment. Studies with different pH-regimes and puffer systems were performed based on OECD 239. Data will be presented with the focus on the comparability to the existing guidelines and effects on *Myriophyllum spicatum*.

MO134

Effect of fluorine on the photosynthetic activity of freshwater and soil algae

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Fluorine can be harmful to humans and ecosystem. Uptake of excessive fluorine can cause skeletal and dental fluorosis to humans, and necrosis and interveinal chlorosis to plants. Fluorine can inhibit efficiency of photosynthesis to plants. Here, we investigated the toxicities of fluorine on the photosynthetic capacity of green algae living in aquatic and terrestrial ecosystems. *Chlamydomonas reinhardtii*, *Pseudokirchneriella subcapitat*a and *Chlorococcum infusionum* were used as test species and sodium fluoride was test material. To investigate the adverse effects of fluorine on the photosynthetic activity of algae, 9 parameters (absorption flux, trapped energy flux, electron transport flux, dissipated energy flux per reaction center, maximum quantum yield of primary photochemistry, quantum yield of electron transport, quantum yield of energy dissipation, average quantum yield of primary photochemistry and blockage of electron transfer from the reaction center to the quinone pool) were measured via Handy Plant Efficiency Analyzer. We observed that fluorine adversely affected photosynthetic function at relatively high fluorine concentrations. These results indicate that fluorine can be harmful to the primary producer of aquatic and terrestrial ecosystem and harass the food web, trophic level and, by extension, health of ecological system

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MO135

Effect of heavy metals on invasive knotweed (Fallopia spp.) and the combined effect of metal pollution and plant colonization on MultiDrug Resistance (MDR) phenotypes in soil bacteria

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The presence of heavy metals in the environment, which is largely due to anthropic activities, represents a stress for all living organisms, impacting ecosystem structure and function. At the microbial scale, the cellular toxicity of metals could alter microbial communities by enhancing MDR bacterial phenotypes through mechanisms of resistance co-selection [1]. Some plant species are able to grow on heavy metal polluted soil; this is the case of the invasive complex *Fallopia* which is able to accumulate metals, leading this plant to be dominant in high polluted urban environment [2]. On the other hand plants are known to be major drivers of soil microbial communities structure and functioning through root exudation [3], and *Fallopia* has been shown to exert significant effects on soil bacterial communities through its secondary metabolites content [4]. In this context we wanted to evaluate the effect of metal pollution on *Fallopia* belowground secondary metabolism, and the combined effect of metal pollution and plant colonization on soil bacterial MDR resistance phenotypes. In this aim, we undertook mesocosm experiments where rhizome fragments of *Fallopia* spp.were grown in greenhouse in soil pot artificially polluted or not with heavy metals (Pb, Zn, Cd, Cr). Our results show that (i) heavy metals delay plant growth at high concentrations but did not affect plant height after 3 months nor aerial part dry weights, only belowground part dry weights were lowered; (ii) each plant genotype could be discriminated on PCA plots at each collecting time, but the effect of metals was only detectable for both belowground plant parts (roots and rhizomes) at 1 month and not at 3; (iii) the antibiotic resistance profiles of soil bacteria were affected by plant or metals but the combined effect of plant and metals was not explained by addition effects, but rather by interaction effects which indirectly confirms the importance of metal-induced change on plant metabolism. [1] J Berg et al. Environmental Science & Technology 44 (2010) 8724-8728. [2] J Soltysiak et al. Acta Botanica Silesiaca 7 (2011) 209-218. [3] S Michalet et al. Plant Physiology & Biochemsitry 72 (2013) 169-177. [4] C Bardon et al. New Phytologist 204 (2014) 620-630.

MO136

Higher tier toxicity testing with microalgae: do light irradiation and species interaction count?

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Single species tests are generally performed under standardized conditions. Although very important to provide first information about the pollutant toxicity, the results of these tests lack to provide information on the potential influence of environmental factors on species sensitivity to the pollutant. In the natural environment multiple variables play a role in determining the effects of pollutants on microalgal species. Contaminants and physical-chemical factors might act as multiple stressors that simultaneously influence algal cells whereas algal species, besides having different sensitivities to the stressors, might positively or negatively interact. In this work copper toxicity to monoalgal and mixed cultures of *Chlamydomonas reinhardtii* and *Synechcystis* sp. was investigated under three different light levels, ranging from low to saturating light. The use of flow cytometry allowed the simultaneous measurement of multiple parameters (growth, chlorophyll fluorescence and oxidative stress) after 24 and 48 hours incubation in monoalgal and mixed cultures. The obtained EC50 values were used to compute Species Sensitivity Distributions (SSD) in order to verify to what extent parameters such as HC5 change in case higher tier tests that take into account high light stress and species interactions are performed. *Synechocystis* sp. resulted to be more sensitive to copper than *C. reinhardtii* under low light intensities. Surprisingly *C. reinhardtii* EC50 shifted to higher values when the alga was incubated under saturating light. Higher EC50 were also obtained when C. *reinhardtii* was co-cultures with Synechocystis sp.. On the other hand no significant changes in EC50 were observed for *Synechocystis* sp. under all tested conditions. Even if some significant differences were observed, overall the tested variables didn't cause considerable changes in EC50 values, as a consequence no significant differences were observed in the HC5 obtained from the computed SSD. In this work the difference in species sensitivity resulted to be more relevant than the changes in sensitivity that might occur for one species in presence of variables levels of light or species interaction. In the optic of using SSD for risk assessment, the choice of a group of species representative of the diversity and sensitivity that characterize the microalgal group might be more important than the realization of higher tier toxicity tests that take into account environmental factors as variable.

MO137

Impact of copper (Cu) and arsenic (As) on the biomarkers of Myriophyllum alterniflorum in an open recirculated system

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For several years, heavy metals have attracted attention due to their abundance and persistence in the environment and their toxicity to human health, animals and plants. *Myriophyllum alterniflorum* DC. (Haloragaceae) is an aquatic macrophyte found in the Limousin rivers (France) notably in the Vienne River whose potential for biomonitoring of metal pollution has been well demonstrated in particular for copper, cadmium and arsenic pollutions. The objective of this study was to identify in this species sensitive biomarkers for the early detection of a river pollution taking into account the hydrodynamic conditions. Oligotrophic synthetic medium with similar composition to the river Vienne was prepared. Watermilfoils were cultured for 21 days with or without contamination by 100 µg/L copper or arsenic in aquariums system (150 L). They were placed in three hydrodynamic zones: quiet, turbulent and laminar flow zone at a rate of 3 cm/s. During this period, physiological biomarkers (respiratory and photosynthetic activities and osmotic potential), biochemical biomarkers (chlorophyll a, b and carotenoids pigments and H_2O_2 content), and morphological biomarkers were recorded. Both contaminants caused an oxidative stress characterized by the generation of hydrogen peroxide, a decrease in the pigments content, osmotic potential, photosynthetic and respiratory activities whatever the hydrodynamic conditions. In addition, a decrease in root length occurs with both contaminants and a decrease in the shoot length only appears during arsenic contamination. This decrease in root and shoot lengths was higher in turbulent than in laminar zone. Therefore, we can consider that biochemical biomarkers as H_2O_2 and pigment content, physiological biomarkers as respiration, photosynthesis and osmotic potential and morphological biomarkers especially as root length were the sensitive biomarkers for Cu and As detection in water. While only morphological biomarkers of watermilfoil were affected by the hydrodynamic conditions. Simultaneously, others biomarkers will be studied like histological biomarker such as micronuleii formation in roots. **Keywords:** *Myriophyllum alterniflorum*, copper, arsenic, biomarker, hydrodynamic conditions

MO138

Interest of using aquatic macrophyte, Myriophyllum alterniflorum, for early stage detection of pollution by copper or arsenic.

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Aquatic macrophytes are important to assess the water quality, notably through the Macrophyte Biological Index in River. They can also be used to fight against pollution. For several years, GRESE laboratory studied watermilfoil to determine its potential use to evaluate environmental contaminations. Alternate watermilfoil, *Myriophyllum alterniflorum*, presents potentially useful biomarkers for the early detection of pollutants but also a significant ability to bioaccumulation. The aim of this study is to identify, in alternate watermilfoil, the most sensitive biomarkers for an early detection of surface water pollution by trace elements such as copper (metal) and arsenic (metalloid). The alternate watermilfoil is cultured *in vitro* for 21 days in a medium contaminated by copper or arsenic at concentrations of 100 and 500 μg.L^{-1}. Several physiological (osmotic potential, photosynthesis, respiration) and biochemical (Glucose6PhosphateDeHydrogenase (G6PDH), malondialdehyde (MDA)...) biomarkers are studied. Simultaneously, the physicochemical parameters of the medium are also investigated and the evolution of the concentration of the pollutant in the watermilfoil tissue is monitored. According to contaminant nature and concentration, biomarkers responses are different. Indeed copper and arsenic reduce differently the respiratory and photosynthetic activities, pigments contents and nitrate reductase activity with a decrease ranging from 10% with copper at 100 μg.L^{-1} to 60 % at 500 μg.L^{-1}; and from 15% with arsenic at 100 μg.L^{-1} to 66 % at the higher concentration (500 μg.L-1). In reverse, G6PDH activity, MDA, proline and osmotic potential, increase from 20% to 70% with the increase in copper concentration. While this increase is more important with arsenic ranging from 30% at 100 μg.L^{-1} to 85% at 500 μg.L^{-1}. Compared to copper, higher impact of arsenic on the biomarkers of *Myriophyllum alterniflorum* is observed. These differences were due to the properties of the two contaminants: one is a trace element (Cu), but becomes toxic above certain concentrations; the other (As) is a non-essential and toxic element. Once the most relevant biomarkers were identified *in vitro*, their responses would be evaluated in *in situ*-reintroduced watermilfoil on sites impacted by urban, industrial or agricultural activities. This study should allow identification of effective tools for biomonitoring of aquatic environments. **Keywords:** *Myriophyllum alterniflorum*, inorganic pollutants, biomarker, biomonitoring.

MO139

Is herbicide toxicity on marine micro-algae influenced by the natural Dissolved Organic Matter (DOM)?

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As primary producers, microalgae can be directly affected by herbicides. These organisms can also be impacted by other environmental elements such as the dissolved organic matter (DOM), that may also interact with pesticides. Indeed, MOD can affect pollutant transport, fate, biodegradation, bioavailability, and toxicity on organisms. Therefore, the present study aimed to investigate whether natural DOM influence the toxicity of three herbicides (irgarol (I), diuron (D) and S-metolachlor (S)), single and in mixture, on two marine microalgae, *Chaetoceros calcitrans* (*Cc*) and *Tetraselmis suecica* (*Ts*). After 6-day exposures to each herbicide (I0.05 and I0.5 μg.L^{-1}; D0.05 and D0.5 μg.L^{-1}; S0.5 and S5 μg.L^{-1}) and to mixtures (M1: I0.05+D0.05+S0.5 and M2: I0.5+D0.5+S5), the effects of herbicides were assessed on growth (doubling time T_D), photosynthetic yield (Y_{eff}), reactive oxygen species presence and intracellular relative lipid content. For each condition, two sets of triplicates were run: one with natural DOM added to the culture media, another without DOM. Without DOM, I0.5 and M2 significantly affected almost all parameters with similar trends in both species: an increase of T_D by more than twice (+125% and +129% for *Cc* and *Ts* respectively, at I0.5), a decrease of Y_{eff} (approximately 30%) and a decrease of the relative lipid content. The M2 impacts observed in *Cc* were similar to the effects induced by irgarol at 0.5 μg.L^{-1}, however a higher toxicity was shown for *Ts* (+167% for T_D), suggesting a possible synergistic effect of mixture. When DOM was added in the culture media, significant effects were also observed with I0.5 and M2 conditions for both species: while the effects of I0.5 in T_D of *Cc* were decreased (+101%), they were strongly enhanced for *Ts* (+178%), as were the effects of M2 (+298%). The presence of DOM decreased the herbicide toxicity for *Cc* whereas toxic effects were increased for *Ts*. These results may partly be due to the complexation between DOM and herbicides, leading to a lower bioavailability of herbicides. However, the difference in toxicity between the two species remains to be explained. Analysis of herbicide concentrations, dissolved organic carbon concentration and DOM, which are still ongoing, will help to improve the understanding of interactions between microalgae, herbicides and DOM. Finally, this study demonstrates the importance to consider DOM as a major factor possibly involved in toxicity modulation in the environment.

MO140

Sensitivity of duckweed species in different test designs

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The sensitivity of the test organism and the test design are important issues in planning ecotoxicological studies. The aim of the study was to demonstrate that the test design affects the sensitivity of aquatic plants. In the study three species of duckweed: the swollen duckweed *Lemna gibba*,thecommon duckweed *Lemna minor*,and the giant duckweed *Spirodela polyrhiza* were exposed to a reference substance 3,5-dichlorophenol in different test designs. A static and a semi static designs, with or without sediment were used. The tests were performed according to OECD TG 221 (2006). The endpoint values were determined on the basis of the frond number and the dry weight. Generated results were statistically analysed. *Lemna minor* and *Lemna gibba* exposed in the static tests were less sensitive, regardless of whether the system was with or without sediment. However, the presence of sediment in the system affects the sensitivity. *Lemna minor* and *Spirodela polyrhiza* seemed to give a similar response, and in the semi-static test without sediment the most toxic impact of the reference substance was observed. *Lemna gibba* appeared to be the most sensitive in the semi-static test with sediment. When comparing these three species, the generated results indicate that *Spirodela polyrhiza* is the most sensitive one. Despite that the model organism used is the floating duckweed, the exposure in various test systems may have implications for ecological relevance to generated results.

MO141

Tissue damage assessment in the macrophyta Lemna gibba exposed emerging contaminants using image analysis

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Emerging contaminants are products that are used daily, an example of these products are mouthwashes. The presence of these compounds in the wastewater is a problem that has been intensifying for 10 years, since in countries like the United States, Canada, Switzerland and France have identified their components in ppm concentrations. Because there is not information on the effect of these products on aquatic organisms, the aim of this study was to evaluate the toxicity 4 mouthwashes (Astringosol, Bexident, Colgate and Listerine) in the macrophyte *Lemna gibba*. Bioassays were performed with a duration of 6 days. Macrophytes (10 colonies) were exposed to 5 concentrations of products (25, 12.5, 6.5, 3.12 and 0.78%) in duplicate. After the exposure time, photographs were taken of each test and analyzed with the software Motic (ver. 2.3), for to evaluate the degree of chlorosis (tissue areas where chlorophyll is degraded) and necrosis (loss of living tissue) of fronds and relate to their degree of lipoperoxidacción. The product that caused the greatest degree of necrosis in the macrophytes was mouthwash Colgate (95.2%) and which had the lowest effect was Bexident (24.7%). The levels of lipid peroxidation have a direct relationship with the degree of necrosis. The analysis of the state of the fronds using the technique of image analysis, it allowed to make the evaluation of damage caused by xenobiotics more accurately, quickly and inexpensively so is a good tool for evaluating effects.

MO142

Toxicity effects of herbicide mixtures on the eelgrass Zostera noltei

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Seagrasses play an important role in coastal ecosystems. Worldwide seagrass declines have been observed due to multiple stressors. One of these stressors is the mixture of pesticides used in intensive agriculture in coastal areas. Effects of mixture toxicity are complex and poorly understood. The knowledge about such effects is limited as the main focus of environmental risk assessment is on single compounds. However, mixture toxicity is reality and thus more ecologically relevant. The aim was to gain understanding about short-term effects of realistic herbicide mixture exposure on the physiology of the eelgrass *Zostera noltei*. For that purpose, macrophytes collected *in situ* were exposed at different concentrations of mixtures simulating the composition of typical cocktails of contaminants in two French coastal lagoons. One experiment was conducted using a mixture of 60% 2,4-MCPA and 40% bentazone representative of the Vaccarès lagoon, Camargue (Mediterranean coast). Another used a mixture of 55% S-metolachlor, 15% atrazine, 15% diuron and 15% cybutryne (irgarol), representative of the Arcachon lagoon (Atlantic coast). Three biomarkers of stress were measured: quantum efficiency of photosystem II, photosynthetic pigment composition and enzymatic activity of glutathione reductase at different time points and total concentrations of herbicide mixtures. Data of the Camargue laboratory exposure experiment were compared with field measurements on photosynthesis and pigment contents. Data showed that environmental realistic mixtures and concentrations had sublethal effects on *Z. noltei*. Photochemical efficiency showed significant decrease at a total mixture concentration of 10 μg/L after 6h or 1 μg/L after 24 h (Arcachon mixture) and at a concentration of 10 μg/L after 24h (Camargue mixture). Effect on pigment composition was detected after 24h. Multivariate analysis of the pigment composition showed primarily a shift in the zeaxanthin:violaxanthin ratio. Field measurements did not show large differences in photochemical efficiency. Field effects were difficult to directly relate to herbicide stress as other stressors were also involved. Understanding effects of realistic chemical mixtures on sublethal endpoints of seagrasses will help to improve ERA and more efficient management strategies to prevent further declines of seagrass meadows worldwide.

MO143

Toxicity of hydrocarbons to blue green algae and higher plants to fulfil SSD requirements

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Following submission of REACH dossiers on petroleum substances, CONCAWE decided to extend the ecotoxicity data base with cyanobacteria and a higher plant species, in response to an ECHA observation that test results on these species were not included in the TLM-model training set, as required by REACH Chapter R10.3.1.3. A number of single hydrocarbons (e.g. toluene, xylene, 1,2,3,4-tetrahydronaphthalene, 1-4-diethyl benzene, methylcyclohexane and 1,2-dimethylcyclohexane) covering a range of hydrophobicity were tested. Toxicity data were generated initially on two aquatic macrophytes (*Lemna gibba and Myriophyllum spicatum)* for toluene and 1,2,3,4-tetrahydronaphthalene, with the remaining four hydrocarbons tested only on *Lemna*. Toxicity data were generated on the blue green algae (*Anabaena flos-aquae*) for all the hydrocarbons. A range of test concentrations of each hydrocarbon were generated by sequential dilution of a saturated solution in test media. Care was taken to minimise loss of test compounds during test media preparation and transfer of test solutions. Toxicity of these hydrocarbons ranged from ~ 1 – 30 mg/L (72h E_rC_{50}) for blue green algae and from ~2- 70 mg/L (7 day E_rC_{50}) for *Lemna* with expected lower toxicity values with an increase in hydrophobicity**.** The two sets of data were well correlated (r^2 = 0.954) Blue green algae were consistently less sensitive (2-3 x) than green algae to these hydrocarbons, and consistently more sensitive (approx. 2-3 x) than *Lemna*.

MO144
Toxicity screening of pesticides towards two marine phytoplankton species
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The PHYTOCOTE project, funded by LabEX COTE, concerns the use of pesticides in Aquitaine vineyards (France), their transfer and impacts on ecosystems. As primary producers, microalgae are at the bottom of the aquatic food web and are of great concern for aquatic ecosystems. In the frame of the "impacts" task of the project, this work aims to develop a screening method to assess and compare the toxicity of several active substances for two marine microalgae commonly used in aquaculture: *T-isochrysis galbana* (chrysophyte)and *Skeletonema costatum* (diatom). The tested pesticides are the herbicides diuron (phenylurea) and S-metolachlor (chloroacetanilide) and the insecticides fipronil (phenylpyrazole) and imidacloprid (neonecotinoid). These screening results will allow us to select substances and species in order to evaluate, at the laboratory scale, the trophic transfer of pesticides from microalgae to a primary consumer (oyster), as well as the induced effects. The screening test consists in 5-day exposures of microalgae cultures to contaminants using sterile 24-well microplates in controlled conditions. Microplate wells are filled with 1.5 mL of sterile culture media and inoculated with cultures (initial concentration of 2×10^4 cell.mL^{-1}). The two microalgae strains are exposed to 5 concentrations of each substance, (3 replicates per concentration and 6 replicates for the control). Growth inhibition, the endpoint selected, is estimated daily by measuring the chlorophyll fluorescence at 685 nm using a microplate reader. At the end of exposure, each well is sampled to determine i) the cell density by flow cytometry and ii) the effective exposure concentration by chemical analysis. EC_{50} are then calculated for each substance and species. These tests are currently ongoing. Preliminary results indicate a variable stability of the substances in the microplate wells within the 5-day exposure, due to plastic adsorption and/or degradation. In our experimental conditions, a linear relationship was obtained between the chlorophyll fluorescence at 685 nm and the cell density measured by flow cytometry. The two microalgae species do not react similarly when exposed to the pesticides. Herbicides are more toxic than insecticides. Finally, this study will provide toxicity data about several substances on the first trophic levels of aquatic ecosystems: primary producers in one hand, and primary consumers exposed by their diet in the other hand.

MO145
Determination of Plant uptake factor (PUF) values with two different crops and two different pH levels in uptake solution under greenhouse conditions
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Plant uptake of pesticides is an important process limiting their availability for leaching, run-off and volatilisation. Chemical hydrophobicity ($\log K_{ow}$) and acid dissociation (pK_a, for ionisable compounds) are the most important properties determining the ratio between the concentration of compound in the plant-root system to that in the pore water adjacent to the roots. The plant uptake factor is used as input parameter in environmental fate mathematical models, such as leaching models (e.g. FOCUS, PEARL). Based on the experimental guideline EUregPUF (**2013**, draft), the standard test system was improved and a ring-test was organized (Lamshoeft et al, **2015**). The plant uptake factor was measured in soil-free hydroponic test systems with intact wheat plants where the root system was incubated in pH 6.5 buffered aerated artificial pore water solution containing a given concentration of the compound. The solutions were continuously aerated and protected from light to avoid algae. By monitoring the cumulative volume uptake and the concentration of the test item in the solution at different time intervals, plant uptake factor values could be calculated. Prior to application, the plants were incubated in non-treated test systems and let to recover. The same test system as for the ring-test was used in this study, with incubation durations of about eight days under greenhouse conditions. As for the ring-test, 1,2,4-triazole was used as test compound. In this study, oilseed rape and maize plants were investigated, together with buffered solutions to pH 6.0-6.5 and pH 7.3-7.8. The plant uptake factor was determined on several sampling dates (e.g. 2, 5 and 8 days after treatment), as well as in the test plants at the end of cultivation, using the equation given by Sweeney (**2014**, submitted).

MO146
Experiments and inverse modeling to plant uptake and degradation of eight emerging organic contaminants
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A myriad of emerging organic contaminants (EOCs) have been detected in reclaimed water. These contaminants are usually not regulated in effluent water. Among them there are many pharmaceutical and personal care products (PPCPs), biocides, and other chemicals used by households and industry. In arid and semiarid countries, reclaimed water is frequently used for irrigation of agricultural crops. Several studies have reported that a number of EOCs are taken up well by plants. Hence, human exposure to those chemicals can be associated with the use of reclaimed water for crops. Several empirical and process-based models have been developed for the prediction of concentrations of EOCs in plants. Accordingly, adsorption to soil, lipophilicity and the speciation of the chemical (neutral or ionized) are the governing properties for the uptake and translocation to edible parts. A mesocosm study in a greenhouse facility was carried out to experimentally determine the uptake of eight EOCs (bisphenol A, caffeine, carbamazepine, ibuprofen, propranolol, sulfamethazine, tonalide, triclosan). Batavia lettuce (*Lactuca sativa* cv. Arena) was grown in a perlite:sand mixture with five concentrations of the eight compounds (0, 12, 29, 58 and 117 μg kg^{-1}). 28 days after starting to water the plants with contaminants, the concentrations in substrate, roots and leaves were determined using gas chromatography (GC) and ultra-performance liquid chromatography (UPLC) coupled to tandem mass spectrometry (MS/MS). Uptake into plants was highest for carbamazepine. Most compounds were rapidly degraded, as seen from the difference in initial and final concentrations within the substrate. Inverse modeling with the standard plant uptake model was used to estimate degradation. Measured dissipation was contrasted with physico-chemical loss (volatilization, translocation, dilution) and the difference was contributed to biodegradation in plant. This indirect method cannot prove degradation but can help to quantify loss processes. Two degradation kinetics were fitted to experimental data, namely first order and Michaelis-Menten. Highest degradation rates in plants were calculated for caffeine (leaves), propranolol (leaves) and ibuprofen (roots and leaves). Of course this method bears some uncertainties but is a first step to consider degradation inside plants, which is widely unknown but essential for predictions of human exposure to EOCs.

MO147
Sorptive capacity of rhododendron leaves for organic pollutants measured using passive dosing: Lipid characterization and passive dosing experiments
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Leaves play an important role in the cycling of semi-volatile organic pollutants as a result of their large surface area and lipid rich cuticle. Semi-volatile pollutants that are scavenged from the atmosphere by leaves can be transferred to the soil through the shedding of waxes and litter [1], where they will either be trapped or re-released to the atmosphere upon decomposition of the plant material. Most multimedia chemical fate models use either an octanol-equivalent model for leaves (e.g., BETR) [2] or reported values from the literature for specific species under the assumption that all plant species have the same sorptive capacity (e.g., CoZMo-POP) [3]. It has been shown, however, that the sorptive capacities of different plant species can vary considerably [4]. In this study we modified an existing passive dosing system to measure the sorptive capacities of the extractable organic matter (EOM) of rhododendron leaves, and characterized the lipids present in the EOM by ^{1}H-NMR spectroscopy in combination with LC-MS and LC-ELSD. While the previous set-up succeeded in reaching equilibrium for cyclic volatile methylsiloxanes within 9 days, the kinetics of equilibration were considerably slower for chlorobenzenes and low chlorinated PCBs. By introducing a fan into the lid of the passive dosing system we were able to enhance the kinetics so that we reach equilibrium as well for dichlorobiphenyls and hexa-chlorobenzene within the same amount of time. This system is used to compare the sorptive capacities of a wide variety of leaves including Norway spruce, douglas fir, red oak, common reed, European beech, rhododendron and the European alder and will provide additional data about variability between plant species that can be compared with existing literature data and that will support

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MO148

Physiological responses of date palm (Phoenix dactylifera L) exposed to air and soil pollution near the industrial complex in Sfax, Tunisia

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This study aims to evaluate the impact of complex air and soil pollutions on date palm plants (*Phoenix dactylifera*), one of the most important commercially valuable crops in Tunisia. These field experiments were performed in the vicinity of the industrial complex in the south of Tunisia (Sfax) for the polluted samples and in unpolluted areas for control date palm plants. Several biomarkers were studied belonging mainly to the antioxidant system like enzymes or antioxidant compounds. Mains results indicate that air and sol pollution induced an oxidative stress as demonstrated by an increase in the rate of hydrogen peroxide (H_2O_2) production, electrolyte leakage and lipid peroxidation in all plant parts. This stress affects chlorophyll and carotenoid contents of leaves. Additionally, the activities of enzymes involved in reactive oxygen species (ROS) detoxification were affected. Air and soil pollution induce an increase in the activities of ascorbate peroxidase (APX), glutathione peroxidase (GPX), superoxide dismutase (SOD) and catalase (CAT). Moreover, in polluted date palm plants the contents of antioxidant molecules like polyphenols and proline increase strongly, this is consistent with the role of these potent antioxidants in scavenging ROS. The present study evidenced the deleterious effects of industrial activity on antioxidative defense system of *Phoenix dactylifera*, an important crop species spreading in arid region of Tunisia.

MO149

Variation of nectar sugar content in different flowering plants

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Pollinators need nectar for survival and provision of the next generation. They choose nectar with higher sugar content and thus higher energy ratio for consumption. There is a lower limit to sugar content where it is not viable for pollinators to visit the respective nectar source again (US-EPA 2012). The EFSA guidance document "on the risk assessment of plant protection products on bees" (2013), provides default values of nectar sugar content for the risk assessment. These worst-case values are the lowest sugar content which may be foraged by the pollinators. The rationale behind these default values is that higher sugar content will result in reduced nectar consumption and therefore a lower exposure to pesticides present in the nectar. The values are based on literature research. The default values given for the risk assessment in crop are 15 % nectar sugar content for honey bees and bumble bees and 10 % nectar sugar content for solitary bees. For weeds and the field margin the default value is 30 %. For the low default value in crop there is the option to refine the risk assessment with experimental data. We present nectar sugar content values from different plant species. Nectar samples were acquired by manual sampling from flowers directly or were collected from honey stomachs of forager bees. We compare the variation in nectar sugar content between the different sampling methods and discuss the distinct sampling procedures. **United States Environmental Protection Agency (2012) White Paper in Support of the Proposed Risk Assessment Process for Bees; submitted to the FIFRA Scientific Advisory Panel for Review and Comment. European Food Safety Authority (2013) Guidance on the risk assessment of plant protection products on bees (Apis mellifera, Bombus spp. and solitary bees). EFSA Journal (2013) 11(7):3295, 266 pp.**

MO150

Variability in Non-Target Terrestrial Plan Studies Should Inform Endpoint Selection

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Inherent variability in non-target terrestrial plant (NTTP) testing is an ongoing issue with respect to the use and interpretation of these data for risk assessment. Statistical differences from control groups are often a product of natural variability, with no apparent biological consequence associated with these differences. Such statistical artifacts can lead to a requirement to conduct multiple concentration/rate studies versus single concentration/rate limit tests, the appearance of non-monotonic dose-response curves which are simply natural variability in the data, or an assumption of risk when there are statistical differences from controls of even a few percent. Standardized NTTP testing protocols were initially designed to calculate ER25 (used in the U.S.) or ER50 (used in Europe) values for various effect measures, including sub-lethal measurements of growth. Recent recommendations for risk assessments to consider 5% effect values (ER05s) in the place of non-definitive NOERs (such as for endangered species assessments) should be approached with caution. Extrapolating to an ER05 can result in values which may simply represent natural biological variability and lead to risk assessment conclusions that may overestimate risk. To address these concerns, we evaluated historical control data from standard seedling emergence and vegetative vigor studies (more than 30 studies of each type). The objectives of this statistical study were to determine the variability of control results across studies for each plant species, examine possible causes for the variation in control results across studies, and, given the inherent variability of controls within each study, determine the percent effect that can be estimated or minimum percent effect that can be detected statistically. Findings indicate that when the control variability is high, only very large effects are statistically significant. The results from this analysis indicate that with current test design and implementation, it will often be impossible to estimate an ER05 and will rarely be possible to estimate an ER10. Furthermore, there is no single ECx that fits all situations because the inherent variability depends on the test type, species, and growth response parameter. This analysis focused on statistical significance only. Biological significance is a separate and important consideration.

MO151

Establishing field studies as a potential higher tier option to refine risk assessment for non-target terrestrial plants

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During the regulatory approval process for pesticides, potential side-effects to non-target terrestrial plants (NTTP) need to be assessed. Routine testing with NTTPs is done in highly standardized greenhouse studies. However, if the obtained results are not sufficient to demonstrate acceptable risk, higher tier testing may be needed. Field studies are frequently mentioned as higher tier options but so far no agreed method has been established. Here we present results of a pilot field study done in order to establish a higher tier method for refining risk assessment for NTTPs. Complexity of NTTP field studies is pointed out and discussed. A homogeneous distribution of plant species in the study field is an important prerequisite for data evaluation in the risk assessment paradigm. Hence, commercially available seed mixtures were evaluated as a potential source for establishing higher tier study sites. In a preceding field study conducted in 2014, it was found that both seed mixture and growth area greatly influence distribution of species. Accordingly, in the present field study conducted in 2015 five different seed mixtures were compared in two distinct locations in Germany with regard to their potential for establishment of a homogeneous species coverage. The two locations differed with regard to soil type, irrigation and amount of seeds sown. To reduce emergence of species from the soil seed bank, soil preparation measures were taken at both sites (herbicide application or ploughing). Vegetation cover was assessed according to an extended Braun-Blanquet scale in four replicates per seed mixture on a monthly basis during the growth season (July to October). Two of the five tested seed mixtures showed acceptable results during the first months of assessment. As the study progressed, ecological processes such as competition and succession became important and will need to be considered in future study design. Emergence of plant species from the soil seed bank has to be reduced in order to establish a homogeneous coverage of plant species. About 20 species emerged from the seed bank, regardless of pre-treatments of the fields which resulted in lack of homogeneity in the study fields. Overall, the field study demonstrated that seed mixtures may represent a viable higher tier option, but there still exist some challenges which we will further examine in order to establish a suitable method for field studies for NTTPs.

MO153

A suggested minimum data list for documenting experimental plant uptake studies

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Quantifying the plant uptake of organic chemicals is critical for assessing human and ecological risks, evaluating plants as biomonitors, predicting phytoremediation effectiveness, and for the continued development and improvement of plant uptake models. Experimental data are commonly expressed as ratios of chemical concentrations in the plant compartment of interest (e.g. shoots, roots, xylem sap) to that in the exposure medium (soil, soil pore water, hydroponic solution). These ratios are generally referred to as bioconcentration factors (BCFs) but are likely not at equilibrium. Experimental methods used to measure plant BCFs have not been standardized and vary widely. This makes inter-study data comparisons tenuous. One of the main difficulties in comparing the results of different studies is the lack of information presented on key parameters needed to compare and reproduce the results, and to assess the quality of the study. This includes information describing the experimental design (number of replicates, appropriate controls), plant growth conditions (light intensity, humidity), chemical exposure (duration, concentration), and the quality control information associated with the methods used for plant and exposure concentration analysis. Large impact is also expected from the plant parameters, such as harvest weight, dry matter content, transpiration, water use efficiency, and growth rate. It is rare that these physiological parameters are assessed. Based on a survey of the literature, simulations with physiology-based plant uptake models [1] and the direct experience of over 20 plant uptake experimentalists, we developed a suggested minimum list of data reporting requirements for experimental plant uptake studies. Moreover, we give advice on how such parameters can be obtained with relatively low efforts. The goal is to understand the observed large variations of results seen from different uptake studies, and ultimately the recommendation of a standard uptake test. **References** [1] Trapp, S. 2015. Calibration of a plant uptake model with plant- and site-specific data for uptake of chlorinated organic compounds into radish. Environ. Sci. Technol. 49(1):395-402 \n *Acknowledgement* - The authors thank the participants of the DTU summer course 12906 in 2015 for the fruitful discussions and contributions.

MO154

Assessing the applicability of a proposed protocol for determining a plant uptake factor (PUF): preliminary results

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Models for estimating the leaching of plant projection products to groundwater incorporate plant uptake as a mitigating process. Transpiration stream concentrations (TSCF) have been the key parameter used in estimating plant uptake. However, there are no standard methods for determining TSCF and considerable variation for a single chemical is often observed in the literature. A new European Crop Protection Association (ECPA) protocol, designed to determine chemical specific plant uptake factors (PUFs) from simple laboratory studies, has been proposed to replace TSCF values. The protocol is designed to calculate chemical specific PUFs from the changes in chemical mass and hydroponic solution volume measured over an eight-day period. Theoretically, PUF values range from 0 (no uptake) to 1 (complete uptake). The objective of this study was to evaluate the feasibility the proposed protocol for determining PUFs using 14C-labeled 1,2,4-triazole (log Kow = -0.58), caffeine (log Kow = -0.07) and diethylphthalate (log Kow = 2.4) and Tybalt wheat as the test plant species. Replicate plant uptake studies were conducted for each chemical. Wheat seeds were germinated in perlite then transferred to aerated hydroponic containers prior to chemical exposure. After adding the test chemicals to the hydroponic systems, measurements of chemical concentration and solution volume made over an 8-day period were used to determine PUFs. The final recoveries and the distribution of 14C were also determined by direct measurement. PUF values for 1,2,4-triazole, caffeine, and diethylphthalate were 0.21 ± 0.04, 1.07 ± 0.23, and 2.61 ± 0.83 respectively. The greater the chemical mass left in solution, the smaller the PUF. However, the PUFs were found to be inversely related to the 14C recoveries that were 95% (1,2,4-triazole), 85% (caffeine) and 52% (diethylphthalate). Volatilization from the hydroponic solution, confirmed in subsequent experiments, was the major loss mechanism for diethylphthalate resulting PUFs much higher than the theoretical maximum. It was also observed in preliminary experiments that high evaporation or transpiration rates make it difficult to determine realistic PUFs. Overall, the proposed PUF protocol is simple to conduct and relatively reproducible. However, additional studies on the impacts of evapotranspiration and chemical volatility are needed before the potential limitations of the protocol can be understood and defined.

MO155

Effect of plant species on the determination of the plant uptake factor (PUF) for use in regulatory fate modelling

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Stakeholders from academia and regulatory authorities have proposed to develop a new study design to determine the plant uptake factor (PUF) for regulatory fate modelling [1]. The German Crop Protection Association (IVA) as well as the European Crop Protection Association (ECPA) together with contract research organizations developed and tested a new study protocol in a round robin test with [^{14}C]-1,2,4-Triazole and wheat plants at pH 6.5. Ten laboratories participated and the results confirmed that a promising new method in a hydroponic system was developed. The updated test protocol was made available at the Piacenza Symposium [2]. For a further implementation of the new procedure with different plants and various pH values, more experiments to determine PUF values of active substances and their metabolites were conducted. Here the analysis of a broad spectrum of studies is presented that were carried out by applying the new study protocol with e.g. potato, tomato, winter oil seed rape and wheat plants. The evaluation of the obtained results focused on the applicability and reliability of the protocol to establish the new method for the use in regulatory fate modelling with various plants.The findings demonstrate that with the new method a robust and reliable test can be performed to determine the PUF with a variety of different crops and agricultural relevant pH values between 5.5 and 7.5. References:\n [1] Hingston, Klunder & Schriever 2013: Report of the EUregPUF Workshop, York, UK. [2] Lamshoeft et al. 2015: First results with a new test design for the determination of a substance specific Plant Uptake Factor (PUF) for use in regulatory fate modeling. Proc. XV Symposium in Pesticide Chemistry. Environmental Risk Assessment and Management (eds. Luzzani et al.), p. 19-20, Piacenza, Italy. http://www.symposiumpesticide.org/wp-content/uploads/2015/06/Proceedings-SPCXV_rev.pdf?e66407

MO156

The plant uptake factor - a regulatory perspective

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In the pesticides risk assessment, regulatory decisions are often based on results of environmental modeling. The input parameters utilized in the calculation of predicted environmental concentrations need to be reliable in terms of precision and accuracy. To meet these requirements, most input values (degradation rate, adsorption to soil particles) are usually derived from experimental studies performed according to accepted guidelines (e.g. OECD) with well established methods developed and validated by scientific experts. The plant uptake factor (PUF) is a parameter describing the ability of plants to take up substances from the soil pore water via their root systems. This specific amount of substance is not susceptible to leaching processes into groundwater. Consequently, the PUF can be a crucial modeling parameter with a high impact on regulatory decision making. This potential of the PUF for refinements of lower tier groundwater assessments has also been recognized by the pesticides industry. Over the last years, considerable effort has been expended by the industry in developing a standard study design for the experimental determination of the PUF. A preliminary study protocol was evaluated in a ring test in ten laboratories in order to obtain information on the robustness, reliability and practicability of the proposed method. In our contribution we will present an analysis and interpretation of the ring test results from a regulators point of view. A profound examination of the uncertainties associated with the PUF will consider variables influencing the PUF as well as experimental quality criteria and the implementation of the PUF in the modeling tools. Finally, we will raise the question what should be defined as “taken up” by plants and provide options for the usage of experimental PUF values derived by a none-OECD test guideline in the groundwater risk assessment for pesticides on a European scale.

Metals in the Environment: Fate, Speciation and Bioavailability in Water, Soil and Sediment (P)

MO157

The role of organic ligand source and type in governing zinc and copper speciation in the Tamar Estuary, UK

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Zinc (Zn) and copper (Cu) are essential elements for the healthy development of all organisms, but uptake in excess can be harmful. Metal bioavailability is governed by chemical speciation, with the free metal ion often being the most readily biologically available and therefore most toxic. Zinc and Cu are now classified as specific pollutants by the UK Environment Agency, and changes to Environmental Quality Standards (EQS) in 2013 under the Water Framework Directive have resulted in revised standards for saline waters being significantly reduced. For Cu, the new EQS of 59.2 nM dissolved (previously 78.7 nM dissolved) now takes into account bioavailability based on dissolved organic carbon (DOC) concentrations, whereas dissolved Zn is now restricted to 612 nM (previously 121 nM) including an ambient background concentration of 17 nM. This change has driven further research to investigate links between metal speciation and potential biological effects. Water samples from the Tamar Estuary (UK) have been collected seasonally over a full range of salinities (~0.1 – 35) over one calendar year. Samples were taken from locations thought to be influenced by different ligand sources (e.g. sediment derived humic and fulvic acids, sewage

effluent, algal blooms). Filtration to 0.4 μm and 0.2 μm was undertaken, and sample DOC, nutrient, and chlorophyll-a concentrations determined. Sample DOC was characterised via excitation-emission fluorescence. The total dissolved and (operationally defined) labile Zn and Cu fraction, complexation capacity (CC), complex conditional stability constants (log K) and free metal ion concentrations have been measured using adsorptive cathodic stripping voltammetry. Size fractionation reveals metal speciation in the freshwater and low salinity regions is controlled via complexation by larger colloids. Total DOC concentrations are not correlated with increased metal complexation, indicating that this parameter may not necessarily be the best indicator of potential bioavailability.
Excitatio-emission data suggests that autotrophic productivity could play an important role in governing [Zn^{2+}] in the high salinity zone.

MO158
Particle structure effects on Chemodynamics: a combined electrokinetic, DLS, SANS and SSCP study
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Chemodynamics studies in environmental systems are intrinsically difficult due to the heterogeneous nature of the natural matrix especially the types of colloidal and particulate ligands present in solution, their sizes and respective structural organization. In the last decade significant progresses have been made in the understanding of the chemodynamics of colloidal dispersions, namely the influence of size and charge effects, conversely our comprehension of the impact of structural effects is still rather incipient. We investigated structural effects on the chemodynamics of metal ions using two well characterized Core-shell particles made of a glassy (PMMA) core decorated by a modified PNIPAM anionic corona, synthesized using either acrylic acid (AA) or methacrylic acid (MA). Their differentiated reactivity leads to the generation of p(MA-N) and p(N-AA) particles with carboxylate charges located, preferentially in the vicinity of the core and at the shell periphery, respectively. The structural characteristics of these particles were addressed over a broad range of pH values (4 to 7.5), $NaNO_3$ concentrations (3 to200 mM) and temperatures (15 to45°C) by DLS and SANS. DLS shows that the swelling of the ~10 to 90 nm thick-particle shells with decreasing temperature, ionic strength or increasing pH is most pronounced for p(N-AA). Potentiometric titration and electrokinetics reflect the easier dissociation of carboxyl groups in p(N-AA) shell. The DLS response of both particles is attributed to the multiresponsive nature of a peripheral, diluted shell while SANS probes only the presence of a quasi-solvent free dense polymer layer condensed on the core surface. Thickness of that layer increases from ~6 to 9.5 nm with increasing temperature from 15 to45°C (15 mM and pH=5) due to the corresponding collapse of the outer diluted shell layer. Overall, the results evidence the microphase segregated shell structure of p(MA-N) and p(N-AA). The binding of Cd^{2+} ions to in p(N-AA) particles was investigated by SSCP and showed an increase in heterogeneity with temperature at pH 7.5 confirming an influence of structural changes upon the chemodynamics. This work illustrates the need of further studies in environmental colloidal and particulate systems to fully understand the effect of structural organization on chemodynamics and demonstrates the utility of characterization techniques, like SANS, to enlighten the link between structure and reactivity in these matrices.

MO159
AGNES at vibrated gold microwire electrode for the quantification of free copper at low concentration levels
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The critical importance ascribed to the free ion in models, such as the free-ion activity model and the biotic ligand model, led to an increasing interest in quantifying this specie in natural media. However, there is only a limited number of techniques able to quantify the free ion concentration with the required selectivity and detection limit. **AGNES** (absence of gradients and Nernstian equilibrium stripping) technique, which was specifically designed and developed to quantify free metal ion concentrations in aquatic systems, seems an ideal technique overcoming most of the limitations of known methods such as potentiometry with ion selective electrode or Donnan membrane technique. Typically, AGNES has been implemented with mercury electrodes and most recently with bismuth electrodes. *Whereas the former gives rise to concerns regarding the toxicity of mercury, the* ***copper speciation*** *is impossible with the latter due to the similarity of their reduction potentials*. Contrarily, **gold electrodes** are well suited for Cu speciation, without the need for oxygen removal, since the metal reduction wave is at more positive potentials than the O_2 wave, avoiding changes in metal speciation. For the first time the application of AGNES at a gold electrode for the quantification of free Cu concentrations is presented. It was found that: i) the amount of deposited Cu follows a Nernstian relationship with the applied deposition potential, and ii) the stripping signal is linearly related with the free metal ion concentration. The performance of AGNES at the vibrating gold microwire electrode (VGME) was successfully assessed for two labile systems: Cu-malonic acid and Cu-iminodiacetic acid at ionic strength 0.01 M and a range of pH values from 4.0 to 6.0. This novel work implementing AGNES methodology at the VGME is of extreme importance showing that there is a highly promising alternative for Cu speciation at the low concentration levels existing in natural waters, with specific advantages coming from: i) the non-toxicity of the working electrode, and ii) the ability to perform measurements without O_2 removal. Furthermore, it highlights the potential of gold electrodes, and other solids electrodes (e.g., Ag, Sb), for the quantification of other free metal ions (e.g., Hg, Sb, As) at low concentration levels. As such, we expect that this new analytical implementation of AGNES will ultimately become an important tool for applications in environmental and toxicological studies.

MO160
Determination of free concentrations of metals with vibrating gold electrodes
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Speciation is key to understanding availability of metals to biota. Hegemonic paradigms such as the Free Ion Activity Model (FIAM) or the Biotic Ligand Model (BLM) highlight the relevant role of free concentrations of the studied substances in the medium for their toxic or nutritional capabilities. So, there is an urgent need for developing analytical techniques able to measure free metal concentrations in all kind of environmentally relevant matrices with sufficiently low limits of detection. The electroanalytical technique AGNES (Absence of Gradients and Nernstian Equilibrium Stripping), with the Hanging Mercury Drop Electrode (HMDE), has proved efficient in measuring free concentrations of elements such as Zn, Cd and Pb in a variety of matrices: seawater, freshwaters[1], humic acid dispersions, soil extracts[2], dispersions of ZnO nanoparticles[3] or CdSe quantum dots[4], etc. However, the use of a mercury electrode implies some drawbacks such as its toxicity. So, there is an active work for the implementation of AGNES with solid electrodes. A first realization has been the determination of free Pb with a bismuth electrode[5]. On the other hand, the Vibrating Gold Microwire Electrodes (VGME) has recently tackled many systems[6]. The aim of this poster is to show recent advances in the application of AGNES with VGME to the determination of free Cu concentrations. Special attention will be paid to unravelling the physicochemical principles (such as underpotential deposition of Cu as a monolayer on the gold electrode or the presentation of mathematical expressions to relate the preconcentration factor with the applied potential), as well as to consider practical issues for its implementation in environmental matrices such as limit of detection, interferences, time of analysis, impact of solution composition, range of linearity, working potentials, and ease of operation.
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MO161
PEST-ORCHESTRA: A tool for optimizing NICA-Donnan model parameters for humic substances reactivity
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Humic substances are a complex mixture of macromolecules that have a high affinity for metal ions and can affect their mobility and bioavailability in the environment. Due to the high heterogeneity of humic substances, their reactivity is difficult to determine, and several models exist to describe their acid-base properties and their complexation to metal ions. Among the various speciation model platforms, the software ORCHESTRA has the advantages to be independent of the operating system and free. Furthermore, it is to be very flexible, since model its model definitions are in text format and thereby accessible and can be modified by users for extensions and modifications. However, until now, no method is available to optimize model parameters using ORCHESTRA. Here we describe the coupling of the PEST parameter optimization software with the ORCHESTRA speciation software, and validate this method as a way to describe humic substances reactivity within the framework of the NICA-Donnan model, by comparing our results with literature

data. The optimization procedure has been applied to obtain optimum NICA-Donnan parameters for the binding of protons and metal ions to Laurentian fulvic acid (LFA). An unconstrained fit gave us proton-binding parameters in good agreement with the results obtained by Milne et al. (2001) on the same dataset using the FIT software. This allow us to validate our approach. Furthermore, this method is applied to optimization of Pb-binding parameters for the same LFA. PEST-ORCHESTRA optimization gives results that are of the same order of magnitude than the ones obtained by Milne et al. (2003) if input parameters are identical (generic fulvic acid proton-binding parameters). However, the optimization procedure yields significantly different values when LFA-specific proton-binding parameters are used as input: $\log_{Pb,1}$ goes from -0.98 to -0.47 and $\log_{Pb,2}$ from 6.55 to -0.43, even if the calculated amounts of LFA-bound Pb are comparable in both cases. This procedure is also applied to derive parameters for Cd- and Zn binding to LFA, which were lacking till date.

MO162

Modelling and determining the varying dissolution rates of sacrificial zinc anodes on pleasure craft on the Hamble estuary, UK and its environmental implications

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This study investigates the physico-chemical parameters within an estuary which affect the dissolution rate of zinc sacrificial anodes on pleasure craft and the impact this has on the aquatic environment. The Hamble estuary is densely populated with around 3000 pleasure craft. Anecdotal evidence suggests zinc anodes decay faster within this estuary compared with other estuaries within the Solent. Zinc anodes are designed and used to protect vessels, fittings on vessels and marine structures from corrosion, but their use can raise zinc concentrations within estuaries, especially around marinas, as they dissolve. Estuaries can therefore sometimes exceed the zinc EQS (Environmental quality standards) limits for the UK of 7.9μg/l causing concern regarding potential impacts on marine life. A survey of boat owners has determined the type of anodes used and possible reasons for a varied dissolution rates within the Hamble. This enables 'hot spots' of anode premature wear to be identified and compared with physical parameters from these areas, which helps determine factors contributing to anode dissolution. Some of the physical parameters suggested include stray electrical currents, salinity variation and the number of boats present. Along with the MAMPEC model and water quality data collected from the Hamble the zinc load from anodes can be determined for individual marinas and the estuary as a whole. Determining these factors and the other main sources of zinc to the Hamble estuary allows the rates of anode decay and the main sources to be spatially mapped and modelled. This will contribute to management plans being re-evaluated and help estuaries such as the Hamble achieve EQS levels.

MO163

ON THE USE OF BULK METAL DEPLETION KINETICS FOR ASSESSMENT OF METAL PARTITIONING DYNAMICS AT BIOINTERFACES

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Assessment of contaminants toxicity toward ecosystems requires the account of chemical species dynamics in solution as well as the integration of the various interfacial processes governing their partitioning and availability at biointerfaces, e.g. internalization, adsorption or excretion. In this work we present an experimental approach that allows the assessment of dynamic partitioning of metals (Cd(II)) at *Escherichia Coli* biointerfaces under poor-complexing medium conditions. The approach is based on the measurement and cautious analysis of Cd depletion kinetics in solution at various biocells number density. In-situ measurements of metal depletion kinetics in the presence of accumulating bacteria were performed with use of electroanalytical techniques. Two mutants of *Escherichia Coli* were selected for the experiments. Genetic mutations on one of the strains led to reduced metal excretion capacity. For the other strain, an additional mutation by plasmid introduction allowed the overexpression of metallothionein in the cytoplasm, which confers upon the bacteria an enhanced capacity to chelate metals in the intracellular volume. Data were quantitatively interpreted on the basis of a recent theory developed by Duval et al[1,2]. in order to quantify the kinetics and thermodynamics of the adsorption, internalization and excretion processes that govern the partitioning of Cd between intra and extra cellular compartments. Doing so, the relevance of the thermodynamic BLM framework could be rigorously evaluated for our system, which contrasts with many metal partitioning studies where the validity of BLM is *a priori* postulated without rigorous justification. **REFERENCES** 1. Duval, J. F. L. Dynamics of metal uptake by charged biointerphases: bioavailability and bulk depletion. *Phys. Chem. Chem. Phys.* **15,** 7873 (2013). 2. Duval, J. F. L. & Rotureau, E. Dynamics of metal uptake by charged soft biointerphases: impacts of depletion, internalisation, adsorption and excretion. *Phys. Chem. Chem. Phys.* **16,** 7401 (2014).

MO164

Understanding Cr biological fate in freshwater bivalves and identification of biomarkers

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Chromium occurs in the environment mainly in two redox forms, namely Cr(III) and Cr(VI). These oxidation states have contrasting chemical and ecotoxicological features. Cr(VI) is of high ecotoxicological concern because it is readily absorbed into cells via the sulfate uptake pathways. Cr(III) is considered less harmful and the actual mechanisms of its internalization into cells are not well known. However, after entering into the cells, Cr(VI) is rapidly reduced to Cr(III) and only Cr(III) will possibly accumulate inside the cells. For this reason, it is desirable to compare accumulation and effects in biological organisms of the two chromium redox forms. Thus, In a first experiment acclimated specimen of the bivalve *Corbicula fluminea* were exposed to four concentrations (0, 5, 50 and 500μg/L) of either Cr(VI) or Cr(III) via the aqueous route for up to 96 h with renewal of exposure medium every 24 h. Bivalves were then dissected to isolate gills and digestive glands. These soft tissues were then digested and assayed for Cr content by ICP-MS. In a second experiment, organisms were exposed using the same protocol and dissected after 24h and 96h of exposure. Chromium content was measured as in experiment one and biomarkers of cellular damage, antioxidant, antitoxic defenses and energetic parameters were also assessed using a Konelab 20 Xti instrument after 24h and 96h of exposure. At 5μg/L, chromium bioaccumulation was comparable to Cr content in control organisms. At 50 μg/L, Cr(III) accumulated to similar levels in the gills and the digestive glands, while Cr(VI) preferentially accumulated in the gills. At 500μg/L, accumulated Cr concentrations were higher in the gills than in the digestive glands for both forms. While Cr(VI) concentration was stable in the exposure medium, total and filterable (0.22μm) Cr(III) concentrations decreased by more than 80% after 24 h. Results of biomarker expressions are currently being collected and elaborated.

MO165

The influence of co-contaminants on the bioavailability of arsenic, lead and cadmium

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Incidental ingestion of contaminated soil is often a major pathway for human exposure to inorganic contaminants. However, exposure is influenced by both biotic and abiotic factors that impact contaminant bioavailability. To date, bioavailability assessment of contaminated soil has focused on arsenic (As), cadmium (Cd) and lead (Pb), however, studies have typically assessed contaminant bioavailability individually, even when multiple elements are present in the same matrix. As a consequence, it is unclear whether interactions between these elements occur within the gastro-intestinal tract (GI tract) which impact absorption and bioavailability. In this study, As, Cd and Pb bioavailability was determined using an in vivo mouse model whereby mice (n = 12 per treatment) were exposed to the contaminant incorporated into AIN93G feed for 10 days. Initially, mice were exposed to each element individually, at three environmentally relevant concentrations; sodium arsenate (1, 5, 10 mg As kg^{-1}), Pb acetate (3, 15, 30 mg Pb kg^{-1}) and Cd chloride (0.2, 1, 2 mg Cd kg^{-1}). Subsequently, binary and tertiary elemental combinations were supplied to exhaust all possible combinations. Contaminant bioavailability was assessed by determining the concentration of As, Cd and Pb in target tissue (liver, kidney, femur) or excreta (urine, faeces). Contaminant relative bioavailability was also assessed in aged (12 years) spiked soils, using individual and tertiary elemental doses. When mice were exposed to As, Cd and Pb incorporated into AIN93G feed, the dose-response varied depending on the contaminant and endpoint assessed. However, for all elements, the dose-response was linear and did not differ significantly for single, binary or tertiary combinations. These results suggest that, at the concentration range tested, there was little or no influence on As, Cd or Pb absorption and bioavailability as a result of co-contaminant interactions. Similarly, when spiked soils were assessed, As, Cd and Pb relative bioavailability was consistent, irrespective of whether the soil contained single or tertiary elemental combinations. Moving forward, research in this area could investigate the potential of interaction occurring between these contaminants at a cellular level.

MO166

Effect of temperature on nickel biodynamics in Daphnia magna as determinated with stable isotope experiment

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Temperature has a strong influence on the physiological state of ectothermic organisms like *Daphnia magna*. The physiological state of the organism can influence metal uptake, detoxification, sequestration and elimination and consequently metal toxicity. The information available about metal uptake rates in *Daphnia* is limited to studies with only one clone and organisms which were not acclimated prior to testing. An acclimation period is necessary in order to achieve the same performance across the different temperature treatments. The objective of the present study was to assess the effect of temperature on nickel uptake in four *D. magna* clones. The uptake of nickel was studied in four *Daphnia magna* clones at 15, 20 and 25°C. Four *D. magna* clones from the same natural population were acclimated during two generations to the temperature treatments. Nickel exposure concentrations were based on the 21 d 50% effect concentration on reproduction of the four *D. magna* clones at 15°C and 20°C, i.e. 50 and 70 μg Ni.L^{-1} respectively plus a control treatment. To determine uptake rates organisms five day old were exposed to the stable isotope ^{62}Ni during 72h and samples were collected at the time points 0, 8, 24, 48 and 72h. To avoid uptake via food organism were not fed during this period. Results showed that nickel body concentrations varied among the four *D. magna* clones. After 48h of exposure nickel concentrations in daphnia were lower at 25°C than at 15 and 20°C. A steady state was generally reached within 24h for all clones at 25°C in contrast with 15°C, which generally only reached a steady state after 48h of exposure.

MO167

Effect of aging on the toxicity of leached rare earth elements in soil over time

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Rare earth elements are increasingly becoming mined and used for various technological advances. As such, the risk assessments of new and existing products containing rare earth metals have been identified as a priority for the Government of Canada's Chemicals Management Plan. Particularly of interest, are soil effect data specific to boreal forested regions where metallic compounds can enter into the terrestrial environment through industrial emissions (e.g., metal smelting, mining, etc.), and ecotoxicological data are lacking. Given that boreal regions form a significant portion (~30%) of Canada's landmass, the effect of rare earth metals were evaluated on boreal species using boreal forest soils spiked with the test substance. To address the effect of leaching and aging on toxicity over time, tests were conducted by simulating deposition and weathering of two earth metals, lanthanum (La) and cerium (Ce), in reconstructed soil columns. Natural soil horizon stratification was maintained throughout the aging and leaching process. Subsequently, the toxicity of the aged soils were evaluated over time on plant growth (*Pinus banksiana*), as well as soil invertebrate reproduction (using the collembolan, *Proisotoma minuta*, and the earthworm, *Dendrodrilus rubidus*). The study was designed to mimic the natural environment, at which metal deposition might occur, with mobilization through the soil profile as as result of, for example, precipitation. The effect of the aging process on toxicity will be discussed, along with a discussion of metal availability by profile.

MO168

The importance of speciation in Chromium hazard and risk assessment

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In aquatic environments, Chromium exists mainly in two redox forms, namely Cr(VI) and Cr(III). The role and the impact of Cr on the environment and living organisms depend on its chemical form. Cr(III) is considered much less ecotoxic compared to the hexavalent form of the same element. However, some recent studies report higher toxicity of Cr(III) than Cr(VI). Experimental verification of speciation during ecotoxicological tests can facilitate comparison among laboratory experiments or between laboratory and field situations. We used IC-ICP-MS to investigate possible conversion between Cr(VI) and Cr(III) in a standardized algal test medium (ISO8692) both in the absence and in the presence of the green alga *Pseudokirchneriella subcapitata*. Aliquots of test medium were spiked with 60 μg L^{-1} of Cr(III) or 115 μg L^{-1} of Cr(VI), corresponding to the EC50s at 72h determined in our laboratory for the two forms. No interconversions between the two redox forms were observed over 72h (i.e., the actual duration of the algal tests) either in the presence or absence of the algae. These results confirm that effects observed in the laboratory can be assigned with certainty to the specific redox form added to the test medium. However, filterable Cr concentrations in solutions spiked with Cr(III) were not stable over the experiment duration (72 hours). Considering that the total (unfiltered) Cr concentrations in the same solutions remained stable over 72 hours, we surmise that the observed decrease in filterable Cr concentrations was due to particle formation and not to Cr losses onto e.g. walls of the flask. Analysis of Cr(III)-spiked aliquots of test medium by Nanoparticle Tracking Analysis (NTA) confirmed that colloidal particles (approx. diameter 150 nm) quickly formed when Cr(III) was added to the medium. Particle diameter determined by NTA did not change with time even if filterable Cr concentrations measured during the experience decreased by 70%. Analogous Similar experiences experiments using Single particles Counting (SPC) system suggest that large particles accumulate in the solution with time, thus making sense of the observed decrease in filterable Cr concentrations.

MO169

Assessing bioavailability of soil arsenic using E. coli whole-cell bioreporters

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We determined the bioavailable arsenic in contaminated field soils before and after soil-washing processes to evaluate the arsenic removal efficiency using novel *E. coli* whole-cell bioreporters (WCBs). All of currently available arsenic bioreporters are based on arsenic responsive genes in *ars* operon, but we used gene in nickel responsive operon (*nik* operon in *E. coli*). Unlike other whole-cell bioreporters, the regulatory protein of *nik* operon, NikR, represses the transcription in the presence of Ni(II). Interestingly, when WCBs were exposed to eight heavy metals and metalloid, it responded only to As(III) exposure and showed the expression of reporter gene (*egfp*) under *nikA* promoter. Moreover, the response was proportional to a dose-dependent manner. Thus, this WCBs would be a genuine bioreporter to determine bioavailable arsenic quantitatively.

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MO170

A meta-analysis on metal uptake kinetics and the relationship between metal accumulation and soil properties in soil organisms

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Bioaccumulation can be a good indicator of the exposure of organisms to metal polluted soils. Metals are taken up from the aqueous phase of the soil (pore water) which is considered the main exposure route for most soil organisms. Metal bioaccumulation is a complex process that includes uptake, internal distribution, storage, and excretion. Toxicokinetics studies can provide a mechanistic understanding of the processes of uptake, elimination, and internal distribution of metals in soil organisms, and therefore also help unravelling the complex processes of metal bioavailability in soil. In this study, metal toxicokinetics in soil organisms and the relationships between metal bioaccumulation, metal exposure concentrations, and soil physical-chemical properties were investigated. We used data from the literature on terrestrial organisms such as earthworms, potworms, isopods, beetles, centipedes, snails, and collembolans. We summarized the uptake and elimination rate constants derived from bioaccumulation tests on different soil organisms. For all species and all metals, bioaccumulation followed a specific pattern which could be described by toxicokinetics models applied to calculate bioaccumulation parameters. Non-essential elements, such as cadmium and lead, generally accumulated in the soil invertebrates with relatively high uptake rate constants and most organisms were able to remove them from the body by excretion. For the essential elements, copper and zinc, internal concentrations remained quite constant for almost all test species. Among soil physical-chemical characteristics, soil pH, cation exchange capacity, clay and organic matter content significantly affected uptake rates of non-essential metals in soil invertebrates. For essential metals, kinetics was hardly influenced by soil properties, but rather prone to physiological regulation mechanisms of the organisms. Our analyses illustrated that toxicokinetics could be a valuable measurement to assess bioavailability of metals in soil.

MO171

Toxicokinetics and toxicodynamics of lead in Enchytraeus crypticus in soil

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The present study aimed at investigating Pb uptake and elimination and toxicty in the potworm *Enchytraeus crypticus* in Lufa 2.2 natural soil. To assess Pb toxicodynamics and toxicokinetics, mortality and internal Pb concentrations were determined at different time intervals during the uptake phase of a toxicokinetics experiment. Body Pb concentrations were also measured at different time points after transfer of the animals to clean soil. To assess Pb availability, total, 0.01 M $CaCl_2$-extractable and pore-water Pb concentrations were measured in the test soil. A one-compartment model was used to describe the uptake and elimination kinetics of Pb in *E. crypticus*, while ultimate LC50 ($LC50_\infty$) and an elimination rate constant for effects of Pb on enchytraeid survival were estimated from the LC50-time relationship. Internal Pb concentrations increased with exposure time and reached steady state after around 7 d. In the elimination phase, body Pb concentrations decreased with time and reached equilibrium after around 4 d. For each Pb exposure concentration, potworm survival decreased with increasing exposure time. For each time interval, mortality of animals increased with increasing exposure concentration. $LC50_\infty$ was estimated to be 446 mg Pb/kg dry soil, which is lower than the 21-d LC50 of 558 Pb/kg dry soil, showing that the toxicity of Pb to *E. crypticus* expressed on total soil concentrations did not reach steady state within 21 d of exposure. LC50 expressed on the basis of internal concentrations in the surviving animals reached steady state within 14 d. It therefore seems that compared to Pb bioaccumulation, Pb toxicity is delayed in *E. crypticus*. The inert Pb fraction in the animals was estimated at 0.17, indicating that around 17% of the internal Pb could not be eliminated. The uptake rate constant was 0.14 g_{soil} g_{worm}^{-1} d^{-1}, and elimination rate constants were 0.96 d^{-1} (based on internal Pb concentration) and 0.046 d^{-1} (based on increasing toxicity with time), suggesting that approximately 5% of the internal Pb contributed to the observed toxic effects. Biological $t_{1/2}$ values for Pb elimination were calculated to be 0.72 d and 15.1 d for toxicokinetics and toxicodynamics, respectively, again indicating that development of toxicity with time was much slower than the uptake of Pb in *E. crypticus*.

MO172

Bioavailability and ecotoxicity of Cu substances to terrestrial organisms

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The Government of Canada's Chemicals Management Plan (CMP) is an initiative created to quantify the environmental and human hazards associated with chemicals used in industry and commerce in Canada. Inorganic metal moieties are one group which has been identified as a CMP priority and form components of larger inorganic and organic substances. Given the volume of chemicals requiring evaluation under CMP initiatives, a metal moiety-based approach has been recommended to stream-line the risk assessment process. However, there is uncertainty over the effect of these moieties of concern to soil systems, and further still, whether other components of the substances (e.g., organic component in the case of organometals) to which they belon are of concern and elicit an effect. In order to assess a moiety-based approach, copper-containing substances, representative of broader groups were selected for ecotoxicity tests: water-soluble (copper sulfate) and water-insoluble (copper oxide) inorganic metals; a water-soluble organic metal salt (copper diethyldithiocarbamate); and water-soluble (copper D-gluconate) and water-insoluble (copper gluconate) organometals. Soil ecotoxicological studies were completed using standardized soil test methods to characterize effect on plant growth (*Trifolium pratense* and *Elymus lanceolatus*) and invertebrate survival and reproduction (*Folsomia candida* and *Eisenia andrei*). Soil chemical analyses were also conducted to evaulate for total metal, total parent compound (in the case of organic metal substances) and ionic activity.

MO173

Exploring the contribution of abiotic and biotic factors in elemental accumulation patterns in the shrew Crocidura russula exposed to air pollution

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Several biomarkers of exposure and effect have been checked in terrestrial small mammals (rodents and insectivores) inhabiting water and soil polluted sites, but less ecotoxicological information is available in air polluted sites. Moreover, the bioaccumulation patterns of abiotic and biotic factors that interact at "real world conditions" were understudied in small mammals. Here, the influence of abiotic (season) and biotic (age and sex) factors in the bioaccumulation patterns of 17 elements with ecotoxicological interest (Pb, Hg, Cd, Fe, Zn, Cu, Cr, Co, Mn, Mo, V, Ni, Sn, Sr, Ba, S, Se) was explored in soft tissues (liver, kidney, lung, heart, spleen, pelt) of Crocidura russula inhabiting a coal-fired power plant impacted area. The importance of the factors analysed was age>season>sex and the shrews from the polluted site showed highest concentrations of all elements in their tissues, specially Pb, Cd, Fe, Sn and Sr. Among tissues, heart and pelt were primary target tissues for accumulation of metals with toxicological interest such as Pb, Mo, V, and Ni. Differential exposure and detoxification response in shrews from polluted site also produced higher dispersion of data. Overall, the power station emissions increase the entrance and/or bioavailability of potentially toxic metals and non-metals in C. russula tissues that bioaccumulate depending on several biotic and abiotic factors. In conclusion, there is necessary the study of such factors to better interpret accumulation data of persistent pollutants and their toxic effects in natural populations.

MO174

Spatial variation of extractable elements in anoxic sediments from the Oslofjord (Norway) and the Gulf of Gdańsk (Poland)

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The distribution of the extractable forms of elements was compared in recently sedimented matter from the Oslofjord (Norway) and the Gulf of Gdansk (Poland). Short sediment cores (20 cm long) were collected at 12 stations, 6 from the Oslofjord and 6 from the Gulf of Gdansk. The sediment samples were analyzed for 62 chemical elements applying the BCR sequential extraction procedure and high resolution inductively coupled plasma mass spectrometry (HR-ICP-MS). Certified reference material (BCR-701, fresh water sediments for sequential extraction) was used for the estimation of the procedure efficiency. Moreover, organic carbon, total nitrogen, stable carbon and nitrogen isotopic composition ($\delta^{13}C$ and $\delta^{15}N$), grain size, ^{210}Pb activity and pigments, were determined in the samples. Spatial distribution of the elements was analyzed with principal component analysis (PCA). Finally, the environmental quality of the sediments was evaluated with contamination factor (Cf), pollution load index (PLI) and risk assessment code (RAC). Multivariate analysis (PCA) separated samples from Norwegian fjord and Gdansk Bay. The most significant component was characterized by high loading values of rare earth elements (REE), that can be explained by metamorphic origin of the Oslofjord bedrock. Relatively high proportion of trace elements Cd, Mn, Pb and Zn were found in the acetic acid soluble extract fraction. This indicates presence of exchangeable, weakly bound forms of these elements, prone to be bioavailable to benthic organisms. On the other hand, Cr, Fe and Ni were found mostly in the residual fraction of the sediments indicating their low mobility and low susceptibility to be released from the sediments. The Cf showed that all locations were contaminated by Cd and Mn with highest values of Cf in the innermost part of Oslofjord (Bunnefjord), where the deeper-water exchange is restricted. This was confirmed by PLI – the highest values of PLI was also found for station located at Bunnefjord. According to RAC, sediments in Bunnefjord and at a station located in a deepest part of Gdansk Bay (Gdansk Depth) were classified as constituting 'very high ecological risk' due to high concentration of Mn in weakly bound acid soluble fraction of sediments. This indicates the potential of Mn to be a threat to local ecosystem ability of this heavy metal to enter food web. High and moderate risks were estimated for Sb and As respectively.

MO175

Scenario analysis of metal fate in estuaries

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Estuaries provide a multitude of ecosystem services such as drinking water supply and water purification. Therefore, estuarine areas have been zones of intense human occupation and industrialisation, with a legacy of historic and current metal contamination. Due to the accumulation of fine-grained sediments, estuaries are sinks for heavy metals which may be problematic pollutants due to their environmental persistence and potential toxicity. Additionally, coastal systems are facing threats associated with climate change that may affect metal mobilization. For example, dredging and coastal squeeze may result in the re-working of tidal flat sediments, while annual river discharge may change (decrease in summer, increase in winter) due to climatic shifts. Such effects will interact with tides and baroclinic circulation and thus determine heavy metal distribution in future estuaries. The present study uses the 3D hydrodynamic model Delft3D-FLOW fully integrated with the water quality model Delft3D-WAQ to investigate environmental dynamics, metal partitioning and metal fate (during 1-year period) in an idealized estuary (funnel-shaped). This model was scaled so that processes could be representative of alluvial systems as the Thames Estuary (London, UK). Simulations were run for Cd, Cu, Fe and Pb under three morphological scenarios (natural tidal flats, moderate coastal squeeze, severe coastal squeeze), three discharge conditions (low, medium, high), and a tidal amplitude of 4 m in comparison to no tide. The results highlight that tidal flats play a significant role in the dynamics of dissolved and particulate metals. Similarly, river discharge and the position of salinity front jointly caused non-conservative metal behaviour. This study underlines the sensitivity of metal partitioning and residence time of contaminants to the abundance of tidal flats, and thus indicates that river-dominated tidal estuaries require these environments to achieve good biogeochemical functions on water purification.

MO176

The effect of environmental conditions on the bioavailability of metals from contaminated sediment

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Abandoned metal mines have left a legacy of sediments contaminated with metals. These sediments may constrain the achievement of the targets set out by the EU Water Framework Directive. A cost-effective approach to dealing with sediments contaminated by metal mining activities should be based on an understanding of how environmental conditions influence bioavailabilty, and hence the conditions under which they present a risk to achieving Good Ecological Status. Here we

have undertaken a controlled laboratory experiment in replicated mesocosms, where we have incubated a field-relevant biomonitor species (*Baetis* spp. mayflies) with sediment collected from a river impacted by an abandoned copper mine. The water (conductivity approx. 40 µS) and *Baetis* were collected from an independent control site of similar geology but not impacted by metal mining. The mesocosms were manipulated to provide three levels of sediment organic matter, three levels of water hardness, and two levels of resuspension, in a fully factorial design. The body burden of metals in *Baetis* was used as a measure of the bioavailability of metals under the experimental treatments. Once set up the mesocosms were incubated at 10 °C for three weeks, for the sediment and water to equilibrate. After this initialequilibration period, a sample of the sediment was collected from each mesocosm for determination of particle size distribution, organic carbon content, and Fe oxides. After the initial equilibration period, the sediment in those replicates to be resuspended was disturbed and allowed to settle. Then 20 individuals of the biomonitor species (*Baetis*) were added and the experimental incubation period began. After incubation for four weeks, the body burden of metals in the *Baetis* was determined using a Vista-Pro CCD Simultaneous ICPOES. At the end of the experiment a second sample of sediment was collected for characterisation as above. Data were analysed using generalized linear models, to establish the effect of the experimental treatments, and any interactions among them, on the body burden of trace metals in *Baetis*: our measure of the bioavailability of metals in the experimental mesocosms.

MO177

A turbidity current plume model for submarine tailing disposal on sloped terrain: providing data for risk assessment of the exposure to pelagic organisms

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Submarine and coastal shallow-water disposal of mine tailings has the potential to spread toxic metal into the aquatic environment. However, the extent of spreading, and therefore also the risk of exposure, depends greatly on the initial conditions of the release: the composition of the slurry, the discharge rate, and the depth and bathymetry around the discharge area. Computer modelling of the disposal plume that takes into account these initial conditions has the ability to predict the transport both to the sea bed and the transport of dispersed matter from the plume in the water column. Therefore, computer modelling of the release can estimate the degree of exposure to pelagic and benthic organisms from mining discharges. Plume calculations in the DREAM model currently handle discharges onto a flat sea bed, taking into account radial spreading and the angle of repose of the deposited matter. However, submarine discharges may be placed on sloped terrain, for example in deep fjords, where the release will cause a turbidity current that will settle according to the bathymetry in the discharge area. Here, we present improvements to the DREAM model to handle density currents by taking into account the bathymetry around the release site. Our approach is to calculate the bathymetric gradient, and simulate the spreading of the turbidity current along the resulting plane, using an estimated roughness to infer friction supplied by the sloping terrain. By applying the improved model to areas of ongoing tailing disposal, we will use the resulting water column concentrations to inform risk assessments of the exposure of metals to pelagic organisms.

MO178

Influence of humic acids on the bioavailability of metals in sediment samples from the Berg River Catchment, South Africa

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Heavy metals in the environment pose serious human health risks, since some of these metals such as lead; mercury and cadmium are highly toxic even at trace amounts. Humic acids are known to be heterogeneous compounds containing mostly carboxyl and phenolic hydroxyl functional groups, providing these organic macromolecules with an important role to play in the transport, bioavailability and solubility of heavy metals in the ecosystem. Humic substances refer to a category of naturally occurring organic materials found in soils, sediment and natural waters. Humic acids are further known to be insoluble in water under acidic conditions, but tend to be more soluble at higher pH values, where they can also be extracted [1-2]. It is known that heavy metals in soil solutions are mostly bound in complexes with low molecular weight organic acids (LMWOA) [3]. The occurrence of these complexes is strongly dependent on pH and some of these complexes formed between heavy metals and LMWOA can be detected with analytical techniques. The work covered in this study is twofold of nature and investigated: (i) firstly, the influence of humic acids on the bioavailability of metals in freshwater sediments; (ii) secondly, the use of spectroscopic analysis to determine the metals in the bioavailable fractions. The different fractions obtained for the use of different humic acids were analysed using inductively coupled plasma atomic emission spectroscopy (ICP-AES) and the concentrations of lead (Pb), nickel (Ni) and copper (Cu) were found to be different in each fraction. The results obtained for the bioavailability of Cd(II), Pb(II) and Ni(II) concentrations in the sediments, have shown that the concentrations obtained at different sampling sites was a function of the organic content, chloride and sulphate concentration. Other seasonal and sampling site physico-chemical conditions also influenced the metal bioavailability in the sediment samples. **Keywords**: Humic acids; heavy metals; sequential extraction; bioavailability; sediments; voltammetry; stripping analysis; Berg River **References** [1] Rahman et al. (2010). Pakistan Journal of Analytical and Environmental Chemistry, 11(1): 42-52. [2] Wang et al. (2010). Chemosphere, 78: 604–608. [3] Wenzel, W.W. and Wieshammer, G. (1995). International Journal of Environmental Analytical Chemistry, 59(2-4): 277 – 290.

MO179

Past and present platinum contamination of a major European fluvial-estuarine system: insights from river sediments and estuarine oysters

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With their massive use in modern societies, Technology-Critical Elements (TCE) are widely dispersed in natural environments requiring a deeper understanding of their environmental impact and cycling in the biosphere. Among these elements Platinum Group Metals (PGM) are modern, technology relevant elements for which the anthropogenic cycle has outcompeted the natural cycles. Stripping voltammetry was used for accurate determinations of platinum (Pt) in historical records of river sediments (dated from 1952 to 2001) and estuarine wild oysters (collected from 1981 to 2013) from the Gironde fluvial-estuarine continuum (SW France) comprising the Lot River. The chronicled samples displayed Pt concentration variations with i) sediment cores from the Lot River showing Thorium (Th) - normalized Pt concentrations up to $11.10^{-5} \pm 0.79.10^{-5}$ for the deepest part of the core which is 5 times higher than the regional geochemical background value (Pt/Th ~ $1.9.10^{-5} \pm 0.39.10^{-5}$) and ii) wild oysters from the Gironde Estuary mouth with Pt concentrations between 0.80 ± 0.01 pmol.g^{-1} and 3.10 ± 0.14 pmol.g^{-1}. These variations reveal the evolution of Pt pressure in this ecosystem with past Pt contamination of sediment cores reflecting the former industrial (smelter) activities in the Lot River watershed and wild oysters having recorded the phasing-out of the smelter-related historical Pt contamination and the recent rise of a new source of Pt to the system. With the application of an empirical model, recent temporal variations of oyster samples were attributed to the extensive increase of Pt demand for car catalytic converters, pointing towards the increasing importance of this emerging source to the aquatic system. These results suggest that anthropogenic vehicle-derived Pt emissions may profoundly affect the Pt budget of the watershed and induce Pt uptake by organisms such as wild oysters that prove to be sensitive biomonitors for Pt contamination over the time. Furthermore this study showed that oysters may bioconcentrate Pt (Bioconcentration Factor, BCF ~ 10^3) and potentially transfer this metal contamination to the higher food chain. Ecotoxicological impacts of such rapid, significant change in the Pt use in industrial applications and its subsequent introduction to the environment are yet still widely unknown and need to be further investigated to complete our knowledge on environmental Pt contamination, processes and possible adverse effects to biota.

MO180

Thallium pollution in Tuscany, Italy: a non-invasive population-based control study

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Here we report the results of a non-invasive population-based study that aimed to quantify Tl levels in 150 urine and 297 hair samples from the population of Valdicastello Carducci and Pietrasanta, Italy. A recent study shown indeed the presence of thallium (Tl) at concentrations of concern in groundwaters near Valdicastello Carducci (Italy). Tl contamination was also found in water intended for human consumption distributed in the same area, where it reaches 9 mg/L Our study aimed to i) quantify Tl with non-invasive sampling procedures; ii) correlate Tl levels found in hair and urine with Tl concentration in the tap waters used by inhabitants; iii) exploit the suitability of saliva as alternative matrix for environmental exposure studies. Tl values found in urine and hair samples were correlated with Tl concentration levels found in tap water in the living area of each citizen and with his/her habits. Tl concentration is higher than 0.6 µg/L and 20 ng/g in about 50% of urine and hair samples, respectively. As Tl is generally removed from the body via urine, the high concentrations of Tl in the biological samples of the studied area must be ascribed to high accumulation of Tl through the food chain. The kinetics of decay of Tl concentration in urine samples was also investigated, finding that even 60 days after the end of the exposition, mean Tl urinary concentration was still extremely high. Our findings indicate that

people resident in the contaminated area significantly accumulated thallium in their urine and hair compared to the reference values of Italian population.

MO181

Uptake and potential acute and physical effects of barite on the pelagic zooplankton Calanus finmarchicus (Crustacea: Copepoda).

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Drilling muds used for offshore oil drilling operations contain weighting particles like barite. The present work involved assessing barite uptake and acute (lethality) and physical (sinking speed) effects of barite-containing drilling mud on *Calanus finmarchicus*. This species is by far the dominating zooplankton species in the Northern Atlantic, and these copepods were treated with an environmentally realistic (10 ppm) concentration of drilling mud up to 168 hrs followed by 100 hrs of recovery. Species were sampled several times during the experimental period, and both microscopic photographs and metal analyses confirmed uptake and accumulation of barite and co-uptake of other metals (e.g. silicon and aluminium). Acute toxicity was assessed, but an LC_{50} was not determined within the range 5-320 ppm. Physical effects of drilling mud on the neutral buoyancy of copepods were assessed by measuring sinking speed of sedated copepods. The main conclusions from this investigation were that i) copepods do in fact filter and ingest mud particles, ii) barite does not appear to have acutely toxic effects, iii) barite particles are withheld within the gut for longer than 100 hrs recovery in clean sea water even though the extractable amounts of Ba, Si and Al were somewhat reduced after recovery, and finally iv) at environmentally realistic concentration ingestion of barite particles affect copepod buoyancy.

MO182

Potential impacts of mine tailing plumes on pelagic filter-feeders

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Deposition of mine tailings in fjords along the Norwegian coast is known to have ecological implications for benthic fauna, but little information is available on the potential impacts on pelagic organisms. Depending on local hydrology, release of tailings can result in plumes where the particle size distribution may extend into the sub-micron range. Whilst the bulk of tailings will settle on the sea floor close to the release point, the sub-micron fraction is likely to remain suspended for longer time periods and for spreading to more extensive. Calanoid copepods, such as *Calanus finmarchicus*, are important pelagic filter-feeders serving as a crucial link in the food chain between primary producers and fish. They are ubiquitously distributed in the Northern Atlantic including fjord systems in Norway. Their impressive capacity for filtering particles for food suggests that they will readily ingest mine tailing particulates, with subsequent exposure to metal-bearing particles and dissolvable metal ions originating from the tailings. We report experimental data for the purpose of environmental risk assessment of mine tailing deposits in Norwegian fiords, where the acquired data will be used to inform risk models. The physicochemical properties of the mine tailing fraction remaining suspended in seawater for an extended period (e.g. 10 d) is comprehensively characterised (composition and particle size distribution). Copepod filtration rates will be determined for different size fractions of particulate matter, sensitivity of copepods to specific elements in the particulate and dissolved phase, and uptake and depuration rates of those elements. Further, the influence of floatation and flocculation chemicals used in mining processes on particle and element bioavailability and potential toxicity will be studied. Finally, we will determine potential implications of environmentally realistic exposures (particle size distributions and concentrations) to mine tailings on copepod survival, growth and reproduction. Environmentally realistic exposure experiments will be designed based on data from field surveys in fjords with active submarine tailing depositions using state-of-the-art in-situ particle imaging systems.

MO183

Determining the removal of platinum group metals in industrial effluent during sewage treatment

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Exposure assessment of metallic substances in industrial effluent discharged to freshwater via sewage treatment plants (STPs) requires knowledge of the retention of the metals during sewage treatment. No information could be identified in the scientific literature on the removal efficiency of municipal STPs processing industrial effluent containing platinum group metals (PGMs). A monitoring study was therefore undertaken to measure the removal of PGMs (platinum, palladium, rhodium and ruthenium) at a number of STPs in order to inform the chemical safety assessment of PGM substances for REACH registration. The removal efficiency for metals during sewage treatment depends upon a number of factors including the sorption characteristics of the metal, the treatment technology applied at the STP and the plant's retention time, which varies according to rainfall and flow rate. A monitoring study was undertaken at three STPs across Europe known to receive effluent containing PGMs. PGMs concentrations (total and dissolved) were measured in influent, effluent and sludge. Analysis was also undertaken for parameters such as pH, alkalinity, hardness and the concentration of total suspended solids. Preliminary analysis of the results indicates differing removal efficiency for the individual PGMs and significant temporal variation at the STPs study sites which is likely to be due to differences in retention time caused by changes in rainfall.

MO184

Use of ombrotrophic peatlands for the assessment of retrospective spatial pollution from atmospheric deposition

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The use of ombrotrophic peatlands as a tool for retrospective quantitative identification of spatial pollution from dry and wet deposition of airborne elements was investigated within the study on the **development of integrated geophysical/ geochemical methods of soil pollution assessment in problematic areas. The study was conducted in Norway and Poland, where six peat bogs in different areas were selected within the possible long-range transport (LRTP) pathways from diverse sources.** From the selected bogs, peat cores were taken, and a comparative analysis of 36 elements was performed along the vertical profiles in layers 3-5 cm thick. Of these, 15 airborne trace elements – proven long range migrants –enriched surface layer of the studied pit bogs to the highest extent. The data showed high correlation with possible emission sources and supplied precise geochemical data related exclusively to the LRTP and element dry and wet deposition. Of the studied sites, N-Sv indicated the highest spatial pollution with Ni (501-716 mgNi/g) and Cu (273-580 mgCu/g). The character of pollution and its concentration in the uppermost 0-3 cm layer unequivocally points to the nickel smelting plant located abroad at the North as a strong contemporary source of these elements. In another peatbog N-Gy at the S of Norway, highly elevated concentrations of practically all airborne elements, with a maximum in the layers 5-10 cm, indicates older LRTP from many sources, which is in **a good agreement with the emission levels in Europe in XXth century, along with the directions of LRTP. In turn, the peat bog P-Iz (Poland) revealed high pollution with Pb, Zn, Hg and As in the layer 10-14 cm. Other sites (P-Wg and N-Mo) i**ndicated temporal pollution from several recent sources, in P-Wg from stronger, and in N-Mo from moderate ones. The samples were dated by the ^{14}C and ^{210}Pb isotope methods indicating the time period of contaminant deposition. The study endorsed peat bogs as reliable tools for quantitative retrospective assessment of LRTP impact, in particular for evaluation of temporal atmospheric deposition of airborne pollutants, comparison of deposition rates in different geographical locations and for estimation of LRTP between different geographic areas. This study has received funding from the Polish-Norwegian Research programme operated by the National Centre for R&D under the Norwegian Financial mechanism 2009-2014 in the framework of Project Contract No. Pol-Nor/199338/45/2013

MO185

High Level Radio-Cesium Pollution in Street Dust Samples Collected in the Vast Area of Eastern Japan and The Probability as a Supply Source to River Systems

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1. Introduction The accident of Fukushima Daiich Nuclear Power Plant (FDNPP) caused severe pollution of radionuclides in Japan. It has been elucidated that significantly higher ^{137}Cs concentrations were found in the litter layer in forests and particle-adsorbed 137Cs is gradually discharged to oceans though rivers. Street dust has been focused for its high concentrations of heavy metals and other anthropogenic compounds. On the other hand, ^{137}Cs level in street dust is not well-known. **2. Materials and methods** Street dust samples were collected from 69 locations within 6.5-240 km from FDNPP in 2013 and 2014. The ^{137}Cs concentration was determined by high-purity germanium semiconductor detector after drying and sieving (< 0.2mm). **3. Results and discussion 3.1. Spatial**

distribution ^{137}Cs concentration in street dust was the highest at 7.6km south (503,000 Bq/kg) and decreased with distance from FDNPP. The higher ^{137}Cs concentrations were shown in the north-west and south followed by north and west in the137Cs concentrations in the 50-150km area revealed south > northwest and southwest > north and west whereas the northeast of Tokyo and the northern part of Gumma Prefecture specifically indicated higher anomaly beyond 150km. The spatial distribution is very consistent with the diffusion path of the radioactive plume estimated by SPEEDI and results of locally conducted on-site monitoring of atmospheric deposition. **3.2. Comparison of ^{137}Cs level with soils and sediments** ^{137}Cs concentrations in street dusts were significantly higher than those in top soils (< 5cm) of forest, paddy, crop, fruits and grassland fields collected in the p< 0.05) ^{137}Cs has been detected in estuarine and bay sediments, and street effluent is a known source of heavy metals entering neighboring water catchment area. These facts indicate a possible washout of ^{137}Cs from street to aquatic ecosystem. **4. Conclusion** Street dust contains high concentrations of ^{137}Cs transported by the radioactive plume. Therefore street dust is an effective media to analyze ^{137}Cs pollution and a probable channel for ^{137}Cs to migrate from land to ocean. It may play a considerable role in elucidating ^{137}Cs dynamics in environment. **Acknowledgement**: We would express our appreciation to the local NPO Corporation and financial support by JSPS KAKENHI (No. 26870180).

MO186

Heavy metal Bio-accumulation in different tissues of Euryglossa orientalis, Chirocentrus nudus and sediments in Bahrekan Bay (The Northwest of Persian Gulf)

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Concentration of Ni, V, Pb and Cu were determined in bottom sediment and liver, gills and fillet of *Euryglossa orientalis and Chirocentrus nudus* along the Bahrekan Bay in the Northwest of Persian Gulf in Iran. Sediment samples and fish species were collected during winter 2013 and spring 2014. Heavy metal analysis was performed by Atomic Absorption Spectrophotometer. Results showed that mean concentrations of heavy metals were high in liver and gills of *Euryglossa orientali*. Also heavy metals had the most accumulation in liver of *Euryglossa orientali*. Target tissue for accumulation Ni,V,Cu and Pb are gills and liver in *E. orientali* and *C. nudus* . In tissues of two fish species fillet has the minimum concentration level of trace elements. The concentrations of heavy metals were lower than legal limits in the fillet (edible part), except for Pb that was higher than permitted limits that is a negative point for consumption by human. Bioaccumulation factors (BAFs) were determined for different tissues of fish species with respect to elemental concentrations in sediment. BAFs results indicated that all BAFs in liver are more than gills and then more than fillet. Also BAF of Cu in liver and gills of k is more than 1.

Toxicity Testing in Sediments - Bioassays As Link Between Chemistry and Complex Benthic Community Testing for Sediment Quality Assessment (P)

MO187

Assessing the predictive ability of Sediment Quality Guidelines (SQG) for fine sediments by using a pollution-sensitive biotic indicator

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Fine sediments are often associated with pollution, because they are a major binding phase for many substances inside waterbodies. The appropriate management of polluted fine sediments, whether concerning, for example, dredging activities or river management plans, is thus an important issue. For prioritization of further assessment or management options for fine sediments, we have developed Sediment Quality Guidelines (SQG) that are based on endobenthic organisms living between fine sediment particles throughout their whole life cycle (Nematoda). Specifically, we first derived Threshold Effect Concentrations (TEC) and Probable Effect Concentrations (PEC) for 40 single substances and 4 sum parameters. Then, for quantification of the toxic potential and subsequent classification of a sediment sample, an index based on the PECs and the corresponding measured concentrations of the sample can be calculated (mean PEC-Q). However, validation of the predictive ability of the index is crucial and thus, we used and independent data set consisting of simultaneously analysed chemical and biological (nematode community) information for this purpose. For each sample of the data set, mean PEC-Q as well as the NemaSPEAR[%]-index (Nematode SPEcies At Risk), which indicates pollution-induced nematode community alterations (Höss et al., 2011, Environ. Int. 37: 940-949), were calculated. The results clearly showed a decrease of the NemaSPEAR[%]-index, i.e. a loss of pollution-sensitive species, with increasing mean PEC-Q. However, the correlation is unsurprisingly not a total one and in specific sediments false-negative and false-positive indications might occur (e.g., due to lower or higher bioavailability of some substances or confounding factors influencing the NemaSPEAR[%]-index). Thus, we finally transformed the correlation of the two indices into "incidences of toxicity" (MacDonald et al., 2011, Arch. Environ. Con. Tox. 39: 20-31), from which the probability of toxicity for a specific mean PEC-Q can be deduced. Specifying the probability of toxicity instead of the mere exceedance or not exceedance of a fixed threshold value likely yields valuable information for risk managers and regulators.

MO188

Toxicity screening of sediments from Lake Geneva using the freshwater ostracod Heterocypris incongruens (ISO 14371).

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Lake Geneva is the largest water body of freshwater in Western Europe, draining a watershed of about 8000 km^2 in Switzerland and France with the Rhone being its major tributary. Lake Geneva is subject to anthropogenic pressures due to a large population, agriculture, and industries, and is periodically monitored for contaminant concentrations in water, sediment and biota [Loizeau et al. 2013]. The last monitoring campaign addressing sediment quality of the whole lake dated back to 1988 and addressed mainly metal and nutrient concentrations in sediments [Arbouille et al. 1989]. More recent studies on the zoobenthos have shown a significant improvement of the quality of sediments over the past twenty years [Lods-Crozet & Reymond 2006]. In 2015, a large sampling campaign supported by CIPEL (International Commission for Lake Geneva Protection) was carried out to provide a comprehensive assessment of micropollutant concentrations in sediments and zoobenthos. We will present the results of testing thirty sediment samples collected from the whole lake, with a sediment-contact test using the freshwater ostracod *Heterocypris incongruens* [ISO 14371-2012]. This test offers many advantages over other traditional sediment-contact tests such as requiring a small sample volume and the possibility to obtain test organisms from cysts then culturing is not needed. Despite the relatively long test duration (6 days) it is a promising method for screening the ecotoxicological quality of freshwater sediments. A preliminary analysis of the results allowed the mapping of the ecotoxicological quality of the sediment, which ranged from no or slightly toxic to highly toxic. We will discuss both the ecotoxicological quality of sediments from Lake Geneva and the performance of this test system. Arbouille D, Howa H, Span D, Vernet JP. 1989. Etude général de la pollution par les métaux et répartition des nutriments dans les sédiments du Léman. Rapp Comm int prot eaux Léman contro pollut, Campagne 1988 : 189-172. ISO 14371-2012. Water quality – determination of freshwater sediment toxicity to *Heterocypris incongruens* (Crustacea, Ostracoda). Lods-Crozet B, Reymond O. 2006. Evolution du zoobenthos profound du Léman. Rapp Comm int prot eaux Léman contr pollut, Campagne 2005 : 141-146. Loizeau JL, Edder P, DeAlencastro LP, Corvi C, Ramseier-Gentile S. 2013. La contamination du Léman par les micropolluants : review de 40 ans d'études. Arch Sci 66: 117-136.

MO189

Implications of freezing sediments for use in ecotoxicological testing under static conditions

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Laboratory-based ecotoxicological approaches for testing sediments often require the use of field samples that have been stored frozen. However, freezing is expected to change the biogeochemical characteristics of the sediment, which in turn may modify the bioavailability of contaminants and thus, the biological responses of the exposed organisms. Therefore, sediment freezing may lead to biased extrapolation of results to environmental conditions. In this study, we monitored over a period of one month the chemical behavior of 1 L static microcosms containing freshly sampled sediment versus the same sediment stored frozen. The results showed that the pH increased differently in the water column during the first 17 days of the experiment, and then reached a similar level in both microcosm types until the end of the experiment. The nitrogen cycle monitored in the overlying water was slower and more intense with the frozen sediment than with the fresh sediment. Toxic species NH4+, NH3 and NO2- were present until day 14 in the water column of the frozen sediment, whereas they were only present until day 4 in the fresh sediment. The concentrations of the N-compounds were close to the toxic threshold values for pelagic and benthic organisms in the frozen sediment microcosms. We thus assume that higher concentrations, i.e. above ecotoxicological threshold, might be reached with sediment containing high biotic activity or if food for the exposed organisms is added to the microcosm. Finally, as expected most of the biota in the frozen sediment died during the freezing period; only bacterial colonies and cyanobacteria grew on the sediment during the experiment. Cyanobacteria, which are known to produce toxins, expanded dramatically between day 14 and 21. In conclusion, we do not recommend stored-frozen sediment for ecotoxicological testing of pollutants under static conditions. However, for method development or bioavailability experiments, when the complexity of natural sediment and experiments need to be conducted over several months, stored-frozen sediment can be used with a minimum conditioning period of 10 to 14 days before introducing the test organisms. For the fresh sediment microcosms, we recommend a 7 days conditioning period before introducing the test organisms. Finally, sediment storage and conditioning should be considered when developing standardized ecotoxicological procedures.

MO190

In vitro screening for steroid endocrine disruptive and Ethoxyresorufin-O-deethylase (EROD) inducing activity in surface sediments from Mar Menor lagoon (SE Spain)

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The Mar Menor (Murcia, SE Spain) is the biggest hypersaline coastal lagoon in the Mediterranean Sea, impacted by intensive agricultural activities, urban growth, massive tourism and historical metal pollution. In this study, we investigated the presence of steroid compounds and Aryl hydrocarbon receptor inducers in surface sediments from the lagoon using *in vitro* bioassays. Fifteen sediment samples from Mar Menor were tested for (anti)estrogenic, (anti)androgenic and EROD activity using the HER-Luc, AR-EcoScreen™ and fibroblast like RTG-2 cell lines, respectively. Organic extracts were prepared from total sediments using methanol (for hormonal, anti-hormonal and EROD measurements) and hexane:acetone (for EROD measurements). Overall, higher *in vitro* activity was detected in sediment extracts from depositional areas of the Lagoon (central and southern basins) and near main marinas. All but two sediment extracts induced EROD activity (maximum value 0.97 ng Eq β-Naphtoflavone/mg sed). Anti-estrogenic activity was also detected in eleven samples, of which five of them were able to block up to 80% of the response caused by EC50 of 17ß-estradiol. Anti-androgenic activity was found in six sediment extracts, of which all but one were able to decrease the effect caused by EC50 of dihydrotestosterone below 80%. None of the sediments extracts induced agonistic estrogenic or androgenic activity. Spatial patterns of *in-vitro* responses obtained in this study were compared with those of *in vivo* responses of sea-urchin pre-embryos exposed to sediment elutriates and with sedimentary organic contaminant concentrations (polycyclic aromatic hydrocarbons, polychlorinated biphenyls, surfactants, pesticides and pharmaceuticals) available from the same sampling cruise and stations. Certain dissimilarities between spatial distribution of chemical concentrations, *in vivo* and *in vitro* responses were observed. Further extensive chemical analysis for contaminant identification is underway to improve the interpretation of our findings. **Acknowledgement** - This work has been supported by Seneca Foundation (Region of Murcia, Spain) through 'BIOMARO' project (15398/PI/10) and by IMPACTA project.

MO191

Analysis of Polycyclic Aromatic Hydrocarbons (PAHs) in Lebanese surficial marine sediments

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Organic contaminants are introduced to the marine environment through coastal oil refineries, shipping activities and accidental spills. The organic contaminants most commonly found in marine sediments are: Polychlorinated Biphenyls (PCBs), Organochlorine Pesticides (OCPs) and Polycyclic Aromatic Hydrocarbons (PAHs). The characterization of PAHs in Lebanese marine sediments is still a new field. The objectives of this research are to i) identify and characterize the concentration of PAHs in the sediments of Tripoli, Jounieh, Dora, and Tyre regions in Lebanon and ii) determine the possible sources of PAHs. Pre-sampling, sediment sampling, lyophilization, Soxhlet extraction, rotary evaporation and gas chromatography were performed. The total PAHs concentrations ranged from 2.49 to 731.93 μg/kg dry weight, with the lowest PAHs concentrations found in Tyre and the highest in Dora and Jounieh. The PAHs were mostly from pyrogenic sources.

Natural toxins: an on-growing challenge for environmental research, monitoring and management (P)

MO192

Harmful algal bloom smart device application: using image analysis and machine learning techniques for early classification of harmful algal blooms

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The Ecological Stewardship Institute at Northern Kentucky University and the U.S. Environmental Protection Agency are collaborating to optimize a harmful algal bloom detection algorithm that estimates the presence and count of cyanobacteria in freshwater systems by image analysis. Green and blue-green algae exhibit different Hue-Saturation-Value color histograms in digital photographs. These differences are exploited by machine learning techniques to train a smart device (cellular phone, tablet, or similar) to detect the presence and amount of cyanobacteria in a small surface portion of a freshwater system. The Harmful Algal Bloom Classification Application (HAB APP) has been field tested and verified to classify both green and blue-green algae. Specifically, the APP has been tested on several small streams and ponds, correctly classifying green algal blooms and has been tested on the Ohio River, correctly classifying blue-green algae in the recent 636-mile long cyanobacteria bloom. The application is planned to be tested and optimized in Lake Harsha, a 22,000-acre reservoir which supplies six million gallons per day of drinking water to the Ohio county in which it lies and is a source of many recreational activities, including swimming, boating, and fishing. The application will be used on images taken from a freshwater intake facility. The presence and amount (or lack thereof) will be verified by other detection instruments and *in vitro* by agency scientists and hysteresis techniques will be used to monitor the presence and amount of cyanobacteria on a periodic (*e.g.* daily, seasonally) basis.

MO193

Global metabolomic characterizations of Microcystis spp. highlights clonal diversity in natural bloom-forming populations and expands metabolite structural diversity

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Cyanobacteria are photosynthetic prokaryotes that are able to synthetize a wild rang of secondary metabolites, with noticeable bioactivity (comprising toxicity) according to the ribosomal and non-ribosomal processes involving numerous NRPS/PKS clusters (Shih et al., 2013). *Microcystis* represents one of the most common cyanobacteria taxa constituting the intensive blooms that arise nowadays in freshwater ecosystems worldwide. These microorganisms produce numerous cyanotoxins (toxic secondary metabolites), which are potentially harmful to Human health and aquatic organisms. With the aim of contributing to better understand the variations in cyanotoxins production, such as microcystins (MC), between clones of the same cyanobacterial blooms, we investigate in the present study the diversity of several *Microcystis* strains isolated from different freshwater bloom-forming populations from various geographical area. Twenty five strains of *Microcystis* from the Paris' Muséum Collection of cyanobacteria (PMC) were compared by an integrated approach combining genotyping and metabolites chemotyping. These characterizations comprise analyses on morphology, Internal Transcript Spacer (ITS), microcystin synthesis gene, and global secondary metabolite contents. Complementary metabolomics shotgun analyses by MALDI-TOF and ESI-qTOF/TOF mass spectrometry reveal clear discriminant metabolic profiles between strains collected from identical or different localities. A global cluster analysis indicates that microcystin synthesis gene presence explains more of the metabolite diversity distribution, than the species or the sample localities do. A global network generated from MS/MS fractionation patterns of the various metabolite detected in all 25 strains performed with GNPS tool indicates that these *Microcystis spp.'* strains produce a wide set of chemically diverse metabolites, comprising only few microcystins, but many aeruginosins and microginins, together with a large set of unknown molecules that still remain to be investigated and characterized at their structure as well as at their potential bioactivity/toxicity levels. Shih et al. (2013) *Proc. Natl. Acad. Sci. U.S.A.* 110:1053-8

MO194

Cyanobacterial water blooms dominated by species from different orders produce teratogenic retinoid-like compounds into surface waters

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It is well known that cyanobacteria produce a diverse spectrum of compounds, some of which can cause adverse effects and pose risk to both aquatic organisms and human. Our study characterizes the presence of retinoid-like compounds in field cyanobacterial water blooms and their production to the surrounding waters. These compounds are important for a lot of physiological processes in living organisms, but also belong to the most potent compounds causing teratogenity. The first part of research focused on testing various processing approaches using solid phase extraction (SPE) for the most effective recovery of retinoid-like mixtures and their concentration from surface waters samples. Extracts of model

cyanobacterial biomasses of common species as well as artificial mixtures of several retinoid-like compounds were used during the optimization process. The total retinoid-like activity was determined by *in vitro* assessment using transgenic cell line P19/A15, the concentration of individual compounds was analysed by LC-MS-MS. Ten distinct procedures were tested for optimization of the sample processing with laboratory samples and model mixtures. Three procedures were selected for processing of samples from field study conducted during summer 2015. Samples of biomass and surrounding water were collected from seventeen independent reservoirs or ponds with massive water bloom distributed throughout the Czech Republic. The detailed taxonomical analyses characterized the composition of the water bloom and revealed dominance of different species. Two of the selected procedures were efficient in concentrating the retinoid-like compounds from field water samples showing comparable results. The water samples from five study sites have shown retinoid-like activity over 20 ng/L equivalent concentration of standard retinoid all-trans retinoic acid (ATRA). The greatest detected retinoid-like activities in water affected by cyanobacterial water blooms reached several hundreds ng ATRA equivalent/L. Study shows that phytoplankton blooms of different composition can produce retinoid-like compounds to aquatic environment. Their production could directly affect the present organisms, especially sensitive early developmental stages, namely in case of massive water bloom development, which is a common problem in many surface waters. The work was supported by the Czech Science Foundation grant No. 14-29370P.

MO195

Influence of natural compounds produced by phytoplankton on the effects of anthropogenic estrogens commonly co-occurring in surface waters

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Adverse endocrine disruptive effects of (xeno)estrogens on aquatic biota have been known for decades. Some of the most potent estrogens like steroidal hormones estrone, estradiol, estriol and ethinylestradiol occur in surface waters world-wide. In addition, other less known chemicals, such as phytoestrogens, can contribute to estrogenic activity. Moreover, overall activity results from complex of both agonists and antagonists functioning of chemicals present in environmental mixtures. Apart from man-made pollutants, wide spectra of chemicals in aquatic environments are produced by harmful algal blooms, including some known phytoestrogens. This study combines results from field sampling with laboratory investigations to evaluate the role of phytoplankton metabolites in estrogenic activity of environmentally relevant mixtures in co-exposure with anthropogenic pollutants. Our research has shown estrogenic activities in biomasses of phytoplankton water blooms of various taxonomic composition from some freshwater reservoirs, where also several anthropogenic estrogens have been detected. The potential influence of the phytoplankton produced compounds on the effects of anthropogenic pollutants has been investigated in detail. The interference with estrogenic receptor has been characterized for extracts of broad spectra of cyanobacterial and algal species from different orders including several widespread species frequently dominating water blooms. The extracts were tested alone and in co-exposure with relevant anthropogenic pollutants that were shown to commonly co-occur with the water blooms. The study represents an example of combination of chemical and biological approaches to investigate less known but widespread sources of estrogens and realistic scenario of mutual interactions of anthropogenic and natural compounds acting in mixtures. The study was supported by the Czech Science Foundation within the project P503/12/0553 and EU FP7 project SOLUTIONS (No. 603437).

MO196

Genotoxicity of complex mixtures of compounds extracted from different strains of cyanobacteria

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The occurrence of cyanobacterial blooms has increased significantly in many regions of the world in the last century due to water eutrophication. These blooms are hazardous to humans, animals, and plants due to the production of cyanotoxins, which can be classified in five different groups: hepatotoxins, neurotoxins, cytotoxins, dermatotoxins, and irritant toxins (lipopolysaccharides). There is evidence that certain cyanobacterial toxins, such as microcystins, nodularins and cylindrospermopsins are genotoxic and carcinogenic. However, the mechanisms of their potential carcinogenicity are not well understood. In our research, we focused on genotoxic effects of complex biomass extracts from laboratory cultured cyanobacteria species. The greatest tested concentration of samples was 2 mg_{DM} /mL (DM – dry mass). However, some of the extracts were cytotoxic at this level, thus genotoxic evaluation was done only for non-cytotoxic levels. The genotoxic potentials of cyanobacterial extracts were evaluated using Single cell gel electrophoresis method (Comet assay) and Cytokinesis-block micronucleus cytome assay (Micronucleus test) , both by using human hepatoma HepG2 cell line. Comet assay reveals single and double strand breaks, and other abnormalities on DNA associated with incomplete excision repair, whereas Micronucleus test is a method for the evaluation of genomic instability. Using Comet assay, positive effects were shown for four cyanobacterial extracts: *Cylindrospermopsis raciborski* at levels 0.2 – 2 mg_{DM}/mL, *Aphanizomenon gracile* at levels 1 – 2 mg_{DM}/mL, *Limnothrix redekei* at level 1 mg_{DM}/mL, and *Microcystis ichtyoblabe* at level 0.2 mg_{DM}/mL. Using Micronucleus test, no positive effects were observed for any of tested cyanobacterial samples (tested at level of 0.2 mg_{DM}/mL, which was non cytotoxic level for all tested samples); positive efects were exerted only by control samples (cylindrospermopsin at level 0.5 μg/mL, Benzo[a]Pyrene at level 2.5 μg/mL, and Etoposide at level 1 μg/mL). Results of our research confirmed hypothesis that complex samples of some cyanobacterial species can exert genotoxic effects, similarly to effects of certain pure cyanotoxins. *This research was supported by the grant Cyanocost COST-STSM-ES1105-24073 and the Czech Science Foundation grant No. GACR P503/12/0553.*

MO197

Toxicity of cyanotoxins to human liver stem cells: effects on 2D and 3D cultures

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Occurrence of cyanobacterial blooms poses a serious threat to the environment and human health due to the production of cyanobacterial toxic metabolites. Microcystins (MCs) and cylindrospermopsin (CYN) are considered to be the most hazardous cyanobacterial secondary metabolites; since they are not only associated with acute toxicity but they can induce also long term adverse effects at chronic exposures. In our study, we investigated hepatotoxic and hepatocarcinogenic effects of cyanotoxins using novel *in vitro* model of HL1hT1 cell line, which represent hTERT-immortalized adult human liver stem cells. HL1hT1 cells are characterized by expression of stem and liver oval cell markers, high proliferation potential, ability of anchorage-independent growth, and lack of gap junctional intercellular communication (GJIC). Micromolar concentrations of CYN induced strong cytotoxic effects in HL1ht1 grown in traditional monolayer (2D) cultures primarily affecting respiration (Alamar Blue assay) and cell energetic metabolism (Neutral Red uptake) rather than membrane integrity (CFDAAM assay). On the other hand, growth and viability of HL1hT1 cells were not affected by MC-LR up to concentration 10 micromol/L. In 3D spheroid culture, HL1hT1 cells became more sensitive to cyanotoxin treatment, where both MC-LR and CYN inhibited formation, growth and viability of spheroids (Alamar Blue) even at concentrations at low as 0.1 micromol/L. The observed increase in the sensitivity of spheroid cultures indicate the ability of liver stem cells to differentiate in 3D microenvironment and to better recapitulate physiological liver-specific gene expression patterns and functions, such as expression of proteins involved in cyanotoxin cellular uptake or bioactivation. This study demonstrates that 3D cultures of adult human liver stem cells HL1hT1 represent a perspective in vitro model which allows studying hepatotoxic, tumor promoting and carcinogenic effects of toxicants and understanding thus the role of stem cells in the development of chronic diseases and toxicities. Support: Project GA15-12408S funded by the Grant Agency of the Czech Republic.

MO198

Uptake and biotransformation of cyanobacterial toxins in cultures of liver progenitor and stem cells

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Cyanobacterial toxins (cyanotoxins) represent important group of aquatic contaminants. Microcystins and cylindrospermopsin are recognized as the most hazardous cyanoxins with respect to their potential for both acute and chronic effects on human or wildlife health. These substances cause primarily hepatotoxic or hepato-carcinogenic effects in mammals, however they might cause negative effects also to other tissue or organ cells, such as kidneys, gastrointestinal tract, neural or immune systems. Organ- and tissue-specific effects of cyanotoxins are most likely governed by differential expression of genes responsible for cyanotoxin cell uptake, bioactivation/biotransformation and elimination, which might dramatically differ among various cell types. In this study, we investigated *in vitro* uptake and biotransformation of cyanotoxins microcystin-LR and cylindrospermopsin in experiments with tissue cultures of rat and human liver progenitor or stem cell lines, namely rat liver epithelial cell line WB-F344 which exhibit characteristics of multipotent liver progenitor cells, and hTERT immortalized adult human liver stem cell line HL1hT1. Sensitive and reliable LC-MS/MS method was used to monitor concentrations of parental cyanotoxins as well as their biotransformation products in the cell culture medium collected from monolayer cultures of undifferentiated and differentiated liver progenitor/stem cells, and also from 3D spheroid cultures of WB-F344 and HL1hT1 cells. Western blot analysis using specific antibodies directed against microcystin-LR was used to detect microcystin-protein adducts in the cells. Using our approach, we were able to characterize ability of each cell line to intake and metabolize microcystin-LR and cylindrospermopsin, to characterize effects of cell culture conditions on the kinetics of toxin uptake and transformation, and to correlate these differences with the changes in expression and activity of proteins responsible for the cell uptake of microcystin-LR (organic anion-transporting polypeptides) and biotransformation of cylindrospermopsin (CYP450). Acknowledgement: Supported by Czech Science Foundation project No. GA15-12408S

MO199

In vitro effects of cyanotoxins on normal and transformed rat liver epithelial cell lines

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Massive proliferations of toxic cyanobacteria (blue-green algae) in aquatic ecosystems deteriorate water quality of freshwaters and have become global environmental problem nowadays. Toxic compounds produced by cyanobacteria pose hazards to wildlife or human health when present in water bodies used as drinking water source or for recreational purposes. Cyanobacterial toxins (cyanotoxins) are divided in chemically and toxicologically different groups, whereas microcystins and cylindrospermopsin are currently considered to be the most hazardous cyanotoxins, since they have been associated not only with acute toxicity, but also shown to induce long term adverse effects at chronic exposures, including liver tumor promoting and/or carcinogenic effects. In this study, we used *in vitro* models based on rat liver epithelial cell line WB-F344, which express markers of liver progenitor-/-oval cells. WB-F344 cells are not capable of anchorage-indepedent growth (AIG), maintain functional gap junctional intercellular communication (GJIC), and they are sensitive to contact inhibition of growth. These non-tumorigenic cells can be neoplastically transformed by ras oncogene. Transformed WB-ras cells are characterized by increase of AIG, downregulated GJIC, reduced contact inhibition of growth, and *in vivo* tumorigenicity. Normal (WB-neo) and ras-transformed (WB-ras) rat liver epithelial cells, were used to study effects of cyanotoxins microcystin-LR and cylindrospermopsin on cell growth and viability in traditional 2D monolayer cultures as well as in 3D spheroid cultures. Antiproliferative/cytotoxic activity of cyanotoxins was strongly dependent on the cell type (WB-neo/WB-ras) as well as on the type of cell culture system (2D/3D). The results indicate that exposure to cyanobacterial toxins provides selective growth advantage to undifferentiated and/or transformed cells over normal differentiated cells. These selective effects occur most probably because of different gene expression patterns and biochemical characteristics between these cell types, possibly including genes involved in toxin uptake or bioactivation. Cell type-specific effects of cyanotoxins might represent an important mechanism leading to disruption of liver tissue homeostasis, and thus contribute to their tumor promoting and carcinogenic effects. Support: Czech Science Foundation Project No. GA15-12408S.

MO200

Innate immunity response to an important freshwater polluting cyanobacterial toxin

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Cyanobacteria are ubiquitous photosynthesizing gram-negative bacteria associated with toxic water bloom in freshwater lakes, frequently serving as source of drinking water. Cyanobacterial production of a wide range of hepatotoxins, neurotoxins and dermatotoxins affects water quality, which is rising environmental and health concern worldwide. One of the most common cyanobacterial toxins are microcystins (MCs). Due to their stability in the environment and high risk to human health, the WHO has established a provisional guideline of 1μg/L for microcystin-LR (MC-LR) in drinking water. Whereas, the hepatotoxic potential of MCs was studied extensively, there is general lack of studies focused on the effects of cyanobacterial metabolites on immunity. The aim of our study was to determine the effects of MC-LR on physiological functions of macrophages (RAW 264.7), which represent one of the key effector immune cells. In our study, we used the concentrations of MC-LR (1 - 1000 nM), which were reported in human blood and thus are considered as relevant. Importantly, we have shown that even nanomolar concentrations of MC-LR significantly stimulated the production of pro-inflammatory cytokines, which could negatively affect human health. Interestingly, we found that the MC-LR-induced changes were not attributed to the inhibition of protein phosphatases activity, due to the lack of microcystin trans-membrane transporters. Considering all pieces of evidence gathered in the present study, we hypothesize that MC-LR is able to directly interfere with macrophage surface receptors that trigger the signaling pathways and thereby alter immune response. These results suggest that MC-LR have significant immunomodulatory activities even in very low and environmentally relevant concentrations. The research was supported by the projects of the Czech Ministry of Education LO1214 and LM2011028, and by the CYANOCOST action (EU COST ES1105). \n \n

MO201

Intracellular and extracellular retinoid-like activity of widespread cyanobacterial species

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Cyanobacteria are common and potentially harmful inhabitants of freshwater worldwide. They are producers of various types of bioactive compounds, which are toxic and they may cause animal death, embryotoxicity, teratogenicity and adversely affect human health. Recent studies indicate that some phytoplankton species can produce retinoid-like compounds that could contribute to some of these effects. Retinoids comprise a family of polyisoprenoid lipids which include vitamin A and its natural and synthetic analogues. These substances act by binding to retinoic acid receptors (RAR), and regulate proliferation, differentiation, apoptosis, cytokine production, gut mucosal immunity and malignant transformation of cells. The aim of our work was to evaluate the variability in production of retinoid-like compounds, which could be released into the environment, by selected widespread cyanobacterial species. We tested *in vitro* potential of extracts and exudates from laboratory-cultured species to induce responses mediated through retinoid acid receptor (RAR) using transgenic murine embryonic carcinoma cell line, which contains reporter luciferase gene under the control of retinoic acid-responsive element. The detailed investigations focused on commonly occurring cyanobacterial species with tendency to form massive water blooms, one coccal (*Microcystis aeruginosa*) and four filamentous cyanobacteria (*Aphanizomenon gracile, Limnothrix redekeii, Cylindrospermopsis raciborskii* and *Planktothrix agardhii*). We analysed 18 extracts and exudates of the five different cyanobacteria. We found activation of retinoid receptor after exposure to intracellular samples from all studied cyanobacterial species. The cyanobacterial exudates of all species except of *P.agardhii* also contained retinoid-like activity reaching up to over 6 μg/l in case of one cultivation of *L.redekeii*. We have recalculated total produced retinoid-like activity using extract and exudate data for each tested cyanobacterial species. The total retinoid-like activity of the five selected species ranged from 0.1 to several μg ATRA/g dry weight. The production of retinoid-like compounds was recalculated to biomass dry weight as well as to cell density. This enables to estimate the production in environmental biomasses characterized by cell density and thus assessment of risks of the produced retinoid-like compounds. The research was supported by the Czech Science Foundation grant No. 14-29370P.

MO202

CYANOCOST: an EU COST Action on Cyanobacterial Bloom and Cyanotoxin Risk Management

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CYANOCOST project includes over 100 active participants from 35 European and neighbouring countries. This EU COST ES1105 Action (www.cyanocost.com) runs from 2012 to 2016 and is focused on the assessment and mitigation of problems presented throughout Europe and beyond by cyanobacteria and cyanotoxins in water resources. Working groups of the action include: (i) collecting information on cyanobacteria and cyanotoxins in European waters and identifying appropriate methods for monitoring and analysis; (ii) assessing data on fate, environmental and health impacts as well as on the economy and wider society; (iii) identifying measures for the reduction of cyanobacterial bloom development by in-lake and catchment methods, and for control of drinking water quality; and (iv) dissemination to end-users and stakeholders in the water resources and environmental sectors, plus the general public. This EU Action aims to disseminate best practices for the monitoring and analysis of cyanobacterial blooms and cyanotoxins, which is supported by four major outcomes of the action (handbooks and special journal issues) that focus on (1) Cyanobacterial monitoring and cyanotoxin analysis; (2) Molecular methods;

(3) Prevention, control and mitigation of cyanobacteria; (4) Drinking water treatment and cyanotoxins.

MO203
Behavioural and physiological responses of the oyster Crassostrea gigas exposed to three strains of Alexandrium minutum producing different toxin types
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Blooms of the dinoflagellate *Alexandrium* spp, producers of Paralytic Shellfish Toxins (PST), are regularly detected on the French coastline. PST accumulate into harvested species, such as the Pacific oyster *Crassostrea gigas*, and can cause strong disorders to consumers at high doses. The impacts of *Alexandrium minutum* on *C. gigas* have often been attributed to its production of PST; however some studies revealed that these algae can also produce extracellular substances, some of which have allelopathic or ichthyotoxic properties. These compounds are excreted in the environment thereby impacting phytoplankton, zooplankton but also marine invertebrates and fishes, without implicating any PST. The chemical identity of those extracellular compounds is still unknown. The aim of this work was to compare the effects of three strains of *A. minutum* producing either only PST, only none-PST extracellular compounds, or both PST and none-PST extracellular compounds, on the oyster *C. gigas*. Behavioural and physiological responses of oysters exposed during 4 days to these three strains of *A. minutum* were therefore compared in order to discriminate between the effects attributable to PST and those induced by extracellular compounds. Our results indicate that the three strains of *A. minutum* induced different behavioural and physiological responses of oysters probably depending on bioactive substances produced by each strain. The extracellular-compound-producing strain primarily modifies valve-activity behaviour of *C. gigas* and induces hemocyte mobilization in the gills whereas the PST-producing strain causes inflammatory responses in the digestive gland. These results suggest that extracellular compounds have a significant harmful effect on the gills, which is the first organ in contact with the extracellular substances released in the water by *A. minutum*. Conversely, the PST impact the digestive gland, where paralytic toxins are released and mainly accumulated, after degradation of algal cells during digestion process of bivalves. This study provides a better understanding of the toxicity of *A. minutum* on oyster, and also highlights the significant role of extracellular bioactive compounds in this toxicity and the need to characterize these substances. Keywords: *Alexandrium minutum*, PST, extracellular bioactive compounds, oyster.

MO204
First insights in the characterization of allelopathic and cytotoxic extracellular compounds from the toxic dinoflagellate Alexandrium minutum
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Harmful Algal Blooms (HABs) result from the rapid increase and growth of toxic microalgae, predominantly dinoflagellates, in marine ecosystems. HABs are of major interest because of their effects upon fisheries and human health. Dinoflagellates from the genus *Alexandrium* have the potential to produce paralytic shellfish toxin (PST) and release unknown extra-cellular compounds (ETC) with cytotoxic, hemolytic, ichtyotoxic and allelopathic activity. These ETC have deleterious effects upon various organisms and specific cell types with poorly described effects. A recent study by our group on physiological responses of oysters exposed to different *A. minutum* strains has revealed that the strain releasing ETC had more deleterious effects than the strain producing PST. This highlights the importance of characterizing the ETC produced by *Alexandrium* spp. as well as their modes of biological action. The nature of these ETC remains a mystery, and the relevant literature is sparse and inconsistent. It is not clear if one or more compounds with different properties are involved in the observed deleterious effects. To begin characterizing the ETC produced by *A. minutum,* we first investigated possible deleterious effects of the compounds on three cell types: diatoms *Chaetoceros neogracile*; and bivalves hemocytes (*Crassostrea gigas* and *Merceneria merceneria*). To explore the chemical nature of putative ETC, we mitigated effects using enzymes assays with protease (trypsin) and a reactive oxygen species (ROS) scavenger (superoxide dismutase). Aqueous filtrate of *A. minutum* impacted the photosystem II activity in *C. neogracile*, as measured by pulse amplitude modulated fluorometry, and this deleterious effects was not mitigated by trypsin. This suggests that the lytic protein-like compounds, previously identified in the literature, might not be the same as those affecting the photosystem II. This highlights that two or more different compounds may be involved in allelopathy. The aqueous filtrate induced a decrease in phagocytosis and in mitochondrial membrane potential, as well as an increase in ROS production in bivalve hemocytes measured by flow cytometry. Superoxide dismutase partially mitigated the effects of the filtrate upon phagocytosis suggesting that ROS were involved in cytotoxicity. Further studies are needed to better characterize the compounds and understand if the allelopathic compounds are also responsible for the cytotoxic effects on bivalve hemocytes.

MO205
ALEXNEXT - Alexandrium sp. analysis by Next-Generation Sequencing
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Harmful algal blooms (HABs) are blooms of microalgae able to produce toxic compounds that are accumulated by shellfish and affect human health through its consumption. Monitoring programs are aimed at avoiding the ingestion of these toxins by forbidding the harvest of shellfish when a HAB outbreaks and/or toxin levels in shellfish flesh are over a legally regulated level. Research and monitoring of marine toxic microalgae tend to reduce the costs and time between the sampling and the results, in order to support decisions that affect human health, food safety and aquaculture and fisheries economy. Shellfish aquaculture is an important economic activity in some coastal areas of Cataloniaand Spain. There have been several closures of Catalan shellfish harvesting areas due to the presence of Paralytic Shellfish Poisoning (PSP) toxins in shellfish flesh since the beginning of the monitoring program in 2002; spirolides were confirmed in 2014. *Alexandrium* species are among the main producers of PSP toxins and spirolides. There are 6 *Alexandrium* species normally detected in Catalan coastal waters, 3 of which produce PSP toxins (*A. minutum*, *A. catenella* and *A. tamarense*), 1 is known to be toxic (*A. preuvianum*) and 2 are considered non toxic (*A. insuetum* and *A. margalefii*). Methods for microalgae identification (microscopy) and toxin detection (e.g. chemical and immunological) are often laborious and expensive. Recently, several genetic techniques have been developed for the study of microalgae diversity; among which, NGS, whose throughput exceeds by millions of times the Sanger sequencing, producing high amounts of data cheaply and spreading the use of genetic analysis to a wide spectrum of scientific disciplines. These characteristics make NGS especially attractive for environmental risk monitoring and public health management. The project ALEXNEXT – *Alexandrium* sp. analysis by Next-Generation Sequencing (TECSPR14-2-0012) is a proof of concept of the suitability of a NGS technique for identifying and quantifying marine microalgae. First, specific primers will be developed and tested on single-species *Alexandrium* samples. Tiered communities based on the DNA or the PCR products of the same *Alexandrium* samples will be made to investigate the limits of detection and the ability to quantify the content of each individual *Alexandrium* species DNA in the sample. Finally, the suitability of NGS will be assessed using selected samples collected from the Catalan coast.

MO206
Cytotoxicity to fish cell lines and endocrine effects of the mycotoxins beauvaricine, deoxynivalenol and ochratoxin-A
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Mycotoxins are natural metabolites of fungal species. They can be especially abundant on grain, and therefore there is increasing concern about their effects for humans and livestock. They can also lead to environmental problems. For instance, they can reach water bodies by leakage or run-off from crop fields affecting fish. Cultured fish can also be exposed to mycotoxins by ingestion of contaminated feed containing grain as a protein source. The toxicity of mycotoxins is well recognized in mammals, but their effects on fish have hardly been investigated. Moreover, in the last years evidence that some of them may act as endocrine disruptors is increasing. The aim of this study was to determine the cytotoxic effects of three mycotoxins, beauvaricine (BEA), deoxynivalenol (DON) and ochratoxin-A (OTA) on two fish hepatoma cell lines (PLHC-1 and RTH-149) and to evaluate their endocrine activities at receptor level. Cytotoxicity was determined by the Alamar Blue, Neutral Red Uptake and CFDA-AM assays after 24 h of exposure to each mycotoxin. For the assessment of androgenic, estrogenic and thyroidal (anti)activities three cell lines permanently transfected with luciferase as reporter gene under the control of hormone receptors were used: AR-EcoScreen cell line with the human androgen receptor (hAR), HER-LUC with the sea bass estrogen receptor (sbER) and PC-DR-Luc cell line with the avian thyroid receptor α (THRα). Results obtained in this work showed that both fish cell lines were very sensitive to the three mycotoxins tested. DON was the most toxic followed by BEA and OTA. None of the three mycotoxins tested activated any of the three types of receptors under study. However, all of them showed anti-hormonal activity, thus being able to decrease the response caused by the effective concentration 50 (EC_{50}) of the corresponding hormone: dihydrotestosterone (DHT), 17β-estradiol (E2) or Triiodotyronine (T3). OTA

(0.02μM) showed antiestrogenic activity, provoking a reduction of the luminiscence induced by E2 EC_{50} (0.07μM). BEA presented anti-androgenic activity and DON and OTA anti-thyroidal activity, at concentrations 1000 times higher than this of the EC_{50} of DHT (0.09 nM) and of T3 (0.09 nM). The data obtained in this work showed a high sensitivity of fish cell lines to the three mycotoxins tested and evidenced that these mycotoxins are endocrine disruptors at receptor level. Acknowledgements - Supported by INIA project RTA2012-00053-00-00

MO207

Surfactants from natural products Itaconic and Fumaric Acids: their ecotoxicity and potential for green chemistry

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Interest in surfactants obtained from renewable sources has increased considerably due to their broad spectrum of industrial and everyday applications, the urgent need of decreasing the environmental impact of various chemical products and the general requirement of a more sustainable chemistry that no longer relies on fossil hydrocarbons. One interesting group of surfactants that can be synthesized in a sustainable manner includes products that can be obtained from fatty amines and itaconic and fumaric acids. Itaconic acid can be obtained from the fungal fermentation of waste biomass and is considered to be non-hazardous and biodegradable. Fumaric acid is a biogenic compound, considered to be in general non-hazardous. A series of different compounds have already been synthesized from these acids and have been evaluated focussing on their physicochemical properties and rheological behaviours. In this work a toxicological characterization of the surfactants was conducted in order to evaluate the safety profile of these products. Acute toxicity tests of 6 different surfactants, all of them having a C12 alkyl chain and most of them a 2-pyrrolidone ring, using *Daphnia magna* were carried out as a first step. Following the OECD guideline 202, young daphnids, aged less than 24 hours at the start of the test, were exposed to the test substance at a range of 7 concentrations for a period of 48 hours. Immobilization was recorded at 24 hours and 48 hours and compared with control values. Results obtained indicated EC_{50} (48 hrs) values ranging from 0.5 mg/L to close to 100 mg/L. The most toxic chemicals are now the focus of chemical modifications which aim either to lower the toxicity observed, or to increase it in view of possible uses as bacterial biocides.

MO208

Comparative toxicity of Quillaja saponins extracts and fractions

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Saponins are natural surfactants, found abundantly in various plant species. Quillaja saponins (extracted from *Quillaja saponaria*) have been approved for use as a type of biofungicide. The extract contains around 70 structurally distinct saponin fractions. Among all fractions, QS-18 is the dominant and most toxic one. Another fraction, the QS-21 fraction, is widely used as an adjuvant. The Quillaja extract contains around 13.5% of QS-18 and 3.7% of QS-21. However, most research so far has been done on the entire plant extract containing all saponins as well as other bioactive compounds. Hence, it is not specifically known where the bioactivity comes from: the saponins or non-saponin content. In addition, as only few of the saponin fractions can be analytically detected, the half-lives in different environmental compartments are rarely known. In this study, the QS-18 and QS-21 fractions were isolated by preparative HPLC. The two isolated fractions will be used in both toxicity and degradation studies. Their toxicities will be compared with the toxicities of the commercially available extracts,Quillaja Saponin S7900® (>10% saponin content) and Quil-A® (>95% saponin content), with 48-h *Daphnia magna* and 30-min *Vibrio fischeri* tests. The relative concentration of the different saponin fractions will be calculated by an internal standard (α-Hederin) using LC/MS. Preliminary results show EC_{50} of Quillaja Saponin S7900® and Quil-A® extracts towards *Daphnia magna* equal to 32.4 ± 1.0 and 19.3 ± 0.6 mg/L, respectively. When the relative concentrations of the different saponin fractions have been determined, the relative contribution of the total and the two fractions can be determined using the assumption of concentration addition. Knowledge of the toxicity of the different saponin fractions together with knowledge of their degradation under different conditions, will contribute considerably to environmental risk assessment of saponin applications.

MO209

Exposure and risks of aquatic organisms to genetically modified corn pollen

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In this study the potential exposure of aquatic organisms to Bt-proteins in Corn pollen has been examined. The objective of this study was to gain insight into the potential exposure of aquatic organisms to Bt-proteins in Corn pollen and estimating the possible effects of this exposure in order to assess whether or not the aquatic ecosystem needs to be considered in the admission of transgenic Bt-corn events in the Netherlands. This research was performed commisioned by COGEM (The Netherlands Commission on Genetic Modification). The research was carried out in phases. The first phase was a literature review to gain insight in the exposure routes and dose-effect relationships. The second phase was a field study to determine pollen deposition and dispersion in surface water. For this purpose, samples were taken in waterways bordering plots with Corn. A total of nine sub locations have been sampled across three areas in the Netherlands. In addition a column test was carried out in order to determine how long pollen of Corn are residing in the water column. In phase 3 the results of the literature study and field study were combined and potential effects on aquatic organisms to Bt-proteins in Corn pollen were estimated. Also, just after the harvest, a visual inspection of the waterways was conducted to verify the assumption that crop residues of Corn in the Dutch situation do not end up in waterways. We have concluded that: It is likely that aquatic organisms are exposed to plant material and pollen of Corn if cultivation takes place adjacent to surface water. In case of cultivation of Bt-Corn potential risks of Bt-proteins for organisms in the aquatic environment are assessed as very low (based on the measurements of pollen deposition, numbers of pollen in surface water and dose-effect relations described in literature. At this moment, based on current knowledge, the aquatic ecosystem does not need to be considered at admission of new GM event of Corn.

MO210

The risk mitigation of E. coli O157 in agricultural environment

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Escherichia coli O157:H7 causes several outbreaks as foodborne diseases by consuming leafy vegetables as lettuce and this pathogen has emerged as very important public health issues because of tthe gastrointestinal illness and long-term, chronic sequelae from an infectionтin many countries including Turkey. Many studies have shown that a definitive relationship between eating of leafy vegetables and human diseases. E. coli originates mainly from animal sources and then transfer to field crops and retail chains. The fate of the species in finishing products that are sold in the local and chain markets was monitored regulary two years long. The samples were inoculated to SMAC Agar and The colonies that grew on CT-SMAC agar and sorbitol negative ones were inoculated to Fluorocult_*E. coli*_O157:H7 agar. The susceptible colonies were examined with pouring 1 ml of Kovacs reagent for confirmation. Latex agglutination tests were made for confirmation. The prevalance of E. coli from lettuce was lower in local markets compared to chain markets. The possible contamination way of lettuce is during irrigation water which pumped from rivers that are contaminated from sewages. the leaves of lettuce become contaminated from manure that are used as fertiliser without waiting enough to decrease the contamination. The next step of this study will be how to prevent the contamination from this source and develop a new methodology for this purposes.

Advances in exposure modelling: bridging the gap between research and application (P)

MO211

How to account for the variability in chemical emissions from consumer products?

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To quantify the environmental risk of the increasing use of personal care products, accurate emission estimates of their components are required. In order to be reliable, these estimates need to consider the variability bound with the use of those products. Variability is induced by economic, demographic and social factors as well as by differences in consumer behaviour. Accounting for this variability is important to identify chemical emission hotspots. Recent (top-down) tonnage approaches estimate chemical emissions through disaggregation at smaller spatial resolution of products sales data using information about population density and GDP. The aim of this work is to explore potential improvements of methods to estimate chemical emissions from consumer products. One possible improvement is the consideration of more variables like age, education level and ethnical origin when disaggregating products sales data at smaller spatial resolution. Another possible approach for estimating the amounts of chemicals released to the environment is to use average product consumption data from consumer surveys thus accounting for differences in consumer behaviour (bottom-up approach). Both approaches define the amount of product used per time which can be expressed in form of a probability density function. In a second step, the inclusion levels of chemicals are derived from the chemical composition of single consumer products. Finally, these values are used to estimate probability density functions of chemicals emitted to the environment. This work focuses on countries for which data is available for laundry products and shampoo, e.g. the European Union, the U.S., India and China. The chemical emission estimation techniques derived from this work can be used to derive global chemical emission maps. These maps can be used both for risk assessment and when conducting life cycle assessments of consumer products.

MO212

Urban modelling for engineered nanoparticle emission estimation: York case study

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Urban pollution has become one of the main threats to human health in developed and developing countries. While half of the world's population already lives in cities, predictions of urban population growth are estimating 2.5 billion more inhabitants by 2050 (UN, 2014). As populations rise in urban areas there will potentially be an increase in the emissions of contaminants to the environment. These emissions will include novel and non-regulated contaminants such as engineered nanoparticles (ENPs). A wide variety of ENPs are already integrated in many different commercialized products such as cosmetics, fuel additives, paints and coatings and will be released to the aquatic environment following use and during disposal (Nowack et al. 2012). These ENPs vary in composition, shape, size and surface coating, which will determine their fate and behaviour after use. At the same time, environmental conditions such as pH, salt composition and presence of natural organic matter will also influences their behaviour and fate. All these factors make the exposure estimations a complex issue. However, the use of models can help us to better understand this complex systems and to fill data gaps where monitoring data is not yet available (Praetorius et al. 2013). In this context the aim of this project was to develop a realistic, small scale model for the prediction of environmental concentrations of several ENPs in urban surface waters. By using local specific data on the use and disposal of commercialised ENP-containing products, jointly with high resolution data of the water chemistry and the physical characteristics of the studied area, we are able to realistically predict the local exposure to these nanomaterials and to estimate their potential impact on urban ecosystems by mapping out exposure hots spots. The high resolution local model for ENP exposure in urban areas was developed by combining two individual but interconnected models: an emissions estimation model and a river model. A multimedia box model approach was used. The emission estimation model includes two main pathways of emission of the ENPs (i.e. via the sewage system and direct run off from field and roads). Emission estimates are obtained at high spatial resolution and are fed into the river model, where different processes, such as hetero-aggregation and sedimentation, are simulated and a final estimate of ENPs exposure is obtained.

MO213

Predicting wastewater treatment plant emissions of pharmaceuticals

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Due to the increased use of human pharmaceuticals, emission of those substances into the environment have been become a major concern. After consumption, pharmaceuticals and some of their metabolites ends up in wastewater and wastewater treatment plants (WWTPs). Not surprisingly, concentrations higher than 0.1 µg/L have been detected in WWTPs. Because many compounds are incompletely transformed in WWTPs, they are discharged into the environment via effluent water and sludge, which forms a potential risk for humans and ecosystems. Therefore, understanding and predicting the fate of pharmaceuticals through WWTPs is important. SimpleTreat is a simple box model that estimate the relative emission of a chemical from a WWTP to the environment. The model has become accepted as an evaluation tool for generic exposure assessment of substances emitted via sewage treatment plants in the European Union. Recently, the model was adapted to ionized substances and provided with new equations for a higher variability in operation parameters. In this study, it is tested whether the fate and elimination of pharmaceuticals are well predicted by the improved SimpleTreat 4.0. Variabilities in predictions are explained. Field data obtained from literature, which are measured in activated sludge WWTPs, were used. This search yielded measured concentrations of 51 pharmaceuticals in 34 different WWTPs. The modelled concentrations of SimpleTreat 4.0 were compared to observed effluent concentrations and chemical emission rates as derived from reported influent concentrations. The model predicts well in a number of cases. However, monitoring data are in some cases contrasting, due to variations in the operating system itself, like additional steps in treatment, which are not included in SimpleTreat. Next to this, differences in detection techniques and the time between sampling and measuring, can cause variability in data, because biodegradation still takes place, which especially can influence the measured effluent concentrations. Furthermore, the chemical-specific data related to ionizing properties of certain pharmaceuticals are uncertain, leading to divergent model outcomes.

MO214

A Model for assessing the fate of contaminants in constructed wetlands

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INTRODUCTION Qatar is an arid country with limited amount of natural freshwater resources. As the population, community infrastructure and economy continue to expand there is a growing need for water resources for many domestic, agricultural, green space and industrial uses. There is currently a heavy demand on desalination of waters from the Arabian Gulf, however, there is value in looking at other potential sources of water to help meet the increasing demand. One such source may be industrial wastewaters that could be treated and beneficially used, particular for non-potable purposes. Produced water from the oil and gas industry represent large volumes of treatable water. At ExxonMobil Research Qatar (EMRQ), we are exploring the potential to use engineered wetlands, also known as constructed wetlands, to treat produced water. There are multiple examples of the use of engineered wetlands to treat industrial wastewater in the Gulf Cooperation Council (GCC) countries. Constructed wetlands typically consist of water flowing through a matrix of soil or sediment substrate consisting of living plants and associated microbes. The systems can be designed in various orientations, such as surface flow or subsurface flow, depending on the desired contaminant treatment and water characteristics. There are a number of biological and chemical processes that result in the sequestration, breakdown, evaporative loss and/or transpiration of contaminants. A significant amount of the activity occurs in the rhizosphere region and depending on the oxic or anoxic state of the system, different biochemical reactions will occur. These systems are designed to maximize removal of contaminants in water flowing out of the system. Consequently, it is important to have a good understanding of the contaminants to be treated and how best they can be treated. An effective approach to help understand constructed wetland behavior and the resultant fate of contaminants is to utilize mass balance models specifically built with the various wetland processes in mind. MASS BALANCE MODEL OF CONSTRUCTED WETLANDS EMRQ, with collaboration of Simon Fraser University (Canada), has developed a simple model for the environmental fate of contaminants in constructed wetlands, with the ultimate goal of aiding in the design and monitoring of engineered wetlands to support treated wastewater use applications. This is a preliminary model that has not been tested with field-generated data and, thus, is applied in an illustrative fashion. Regardless, the model is useful as an evaluative tool for exploring the potential treatment of different types of contaminants, ranking treatment effectiveness among contaminants groups, and identifying parameters or processes that are important to understand in more detailed to build more representativeness into the model. With time and application to field data, the model may be used to help design similar systems, understand their inner workings and also potential mitigations required to ensure the safe and effective use of the wetland across its lifetime of operation. The model has been developed with modules for: (i) environmental distribution of contaminants in aquatic system; (ii) uptake, translocation and biotransformation of contaminants in vegetation; (iii) bioaccumulation in aquatic biota of wetlands; and (iv) risk evaluation of contaminant toxicity to resident biota. The model computes both steady-state and time dynamic results of the mass and concentrations of contaminants in the wetland. The wetland model is currently able to simulate free surface flow, horizontal subsurface flow and vertical subsurface flow wetland configurations. Model inputs include wetland characteristics (e.g., compartment volumes, dimensions, organic carbon content, biotic growth rates); environmental conditions (e.g., water flow and temperature); and contaminant properties (e.g., molecular weight, degradation half-lives). MODEL ASSUMPTIONS This model is based on some key assumptions. First, it is assumed that the chemical is homogeneously distributed within each of the wetland media (e.g., water, sediments, plants) and that the wetland media can be described as single compartments in its exchange with its surrounding environment. It is recognized that this is an over simplification and that in particular there is likely to be a significant concentration gradient within the different media near the water intake location compared to those same media nearest the outfall. Realistic contaminant exchange between media will be further complicated by this effect. However, for our purposes as stated above, this is a reasonable assumption to make for such a preliminary model. A second key assumption is that the rhizomes are part of the vegetated rooting medium and in equilibrium with the rooting medium. This assumption may be reasonable for many organic contaminants and nonessential metals but not for essential nutrients such as nitrogen and phosphate. The model further assumes that vegetation can increase in biomass, which reduces the chemical concentration but causes no net loss of the chemical from the wetland. While these assumptions may be clearly gross simplifications of reality, they are reasonable first approximations for this screening level tool. **\n** MODEL APPLICATION To run the mass balance model for each of the wetland types mentioned above, the user must first identify the system dimensions, particularly the surface area (footprint) of the various media, water and sediment compartment depths and intake wastewater flow rate. There are a number of other process specific parameters (e.g., media diffusive exchange rates, media biodegradation rates, etc.) that need to be specified. At the moment assumptions have been made about these parameters on the basis of other studies. In some cases the values associated with constructed wetlands may be quite different from what is seen in nature and thus this adds another layer of uncertainty to the model. With time, addressing the more accurate representation of these values will be important for real world applications. Engineered wetlands are commonly designed to accommodate a combination of species of submerged, emergent, and floating vegetation. This promotes stronger microbial diversity by allowing vegetation coverage over different water depths. Typically, emergent species are the most common vegetation species in wetland design because they are involve in most of the processes in constructed wetland. Some of the key parameters associated with these plants will be surface area coverage density, growth rates, contaminant update, removal processes and storage capacity. Again, these and other plant input data will be based on previous studies. This may be particularly important for our application in Qatar as the climate conditions are likely to result in extreme representations of these parameters. Field-based plant-specific studies will be needed in the future to help derive more realistic values for our purposes. In addition to plant uptake and fate transport processes, the model also includes the functionality to include other lower order organisms (e.g., phytoplankton, zooplankton, benthic invertebrates) and fish to determine how contaminants may have an impact in the wetland system. This bio-uptake / bioaccumulation module draws upon extensive experience in the field of food web dynamics and ecotoxicology and provides another interesting layer of detail to a system that is known to become relatively diverse in nature and also depend on some of these organisms to maintain its health and effectiveness. Finally, the model has been developed to examine a suite of oil and gas relevant contaminants. Representative constituents of volatile, semi-volatile and polycyclic aronmatic hydrocarbons can be modeled. It is possible to examine other ionizable organic compounds such as Naphthenic acids. Metals can also be modelled in the system. In each case the model will require information on the physicochemical attributes of constituent that will help determine its ability to transport between media and be sequestered or transform in a media. Ecotoxicity data will be necessary to determine at what concentrations of each constituent effects on microbes, plants and other organisms may be seen. REPRESENTATIVE ANALYSES Three representative wetland orientations (free surface flow, subsurface vertical flow and subsurface horizontal flow) were modelled using a non-ionizable organic, an ionizable organic and a metal constituent to show the functionality of the mass balance modeling tool. Differences in the ability of each wetland to remove each contaminant from the wastewater stream can be expressed. The removal fraction of each constituent is estimated for the various processes, both at steady state and with time using the dynamic analysis. The steady state analysis provides a good indication of the anticipated removal efficiency in the wetland and the ability to quickly compare removal processes across contaminant constituents and across different wetland systems. This is quite helpful for comparison and ranking purposes. The temporal simulation provides the benefit of predicting the time-variable loading of constituents in media which may provide some indication of the timing required for remedial action in the system (e.g., plant harvesting once the holding capacity is met and removal efficient significantly declines; metal toxicity to microbes in the sediment/rhizosphere). This too is beneficial specifying the design of the system and determining the cost-benefit analysis of different approaches and systems. CONCLUSION Because there are currently insufficient data to conduct an actual model performance evaluation, the model has only been applied in an illustrative fashion. This application aims to demonstrate some of the key model features. The model may be most useful in evaluating the relative treatment capacity of wetlands for certain contaminants where certain contaminants are removed efficiently while others will be largely unaffected by wetland treatment. The model can also illustrate the relative importance of various chemical removal mechanisms for particular contaminants and illustrate the relative capacity of wetland treatment for different contaminants depending on substance and wetland properties. Also, model results provide insights into the temporal response of contaminant concentrations in wetland media and resulting contaminant concentrations in different wetland media. Finally, it may help to assess if the buildup of concentrations in the wetland media can pose ecotoxicological concerns for resident macrophytes, micro-organisms, invertebrates and fish that inhabit the wetland. Such evaluations are regularly used in environmental assessments of commercial chemicals and may also be useful in assessing the potential of wetlands to treat contaminated wastewaters. The ability of wetlands to treat contaminated wastewater is highly dependent on the contaminants and wetland characteristics. While some contaminants will be efficiently removed from wastewaters by the wetlands, others will be minimally affected. The models may therefore be useful in the planning and design of engineered wetlands. The models can make estimates of contaminant removal efficiency of the wetlands throughout their lifetime and predict whether wetland treatment can be expected to produce effluent streams that meet health and environmental standards. They can also be used to investigate key wetland design specifications such as size and vegetation type and flow patterns. Furthermore, they may also help indicate if certain chemicals in the wastewater stream will need special treatment approaches to affect their removal. It is important to emphasize that this is a preliminary model that has not been field tested. Currently, confidence in the model results is based on past applications of similar models to assess the multimedia environmental distribution, exposure and risk of contaminants in other contexts than engineered wetlands. These experiences provide a rationale for further testing and refining of the engineered wetland model presented in this study. With future efforts in model parameterization and performance analysis, the model may become more useful as a predictive tool for assessing the remediation capacity of contaminants using various engineered wetland designs. It is recognized that future work should include the evaluation and testing of the model capability to predict these various scenarios using field data. This type of assessment would help highlight gaps and future areas of study. Subsequently, this tool should provide valuable insights that can guide further studies for designing and monitoring the effectiveness of engineered wetlands for wastewater reclamation purposes. **\n**

MO215

Human exposure to household surface cleaning products: Application of a two-field model

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Indoor cleaning activity is a major human household activity and cleaning products contain volatile organic compounds (VOCs) that constitute a potential threat to indoor air quality and occupants health [2]. The overall objective of this study is to evaluate the short-term human exposure to household cleaning products. More specifically we aim 1) to establish a dynamic modelling framework which accounts for the near-person inhalation and dermal exposure to indoor cleaning products; 2) to determine the evolution of chemical mass and concentration associated with indoor cleaning activity, and to determine the key factors affecting chemical fate and exposure for indoor environment; and 3) to determine the short-term human intake, intake fraction (iF), and product intake fraction (PiF). We modify a two-zone model to describe the near-person and the far-person field identifying four transfer compartments, i.e. near-person surface, near-person air, far-person surface, and far-person air. Transfers between compartments are described by first order transfer rate constants structured in a matrix (K matrix) to describe the mass flows between different. The exposure matrix (XP matrix) relates the mass in a given environmental compartment to the intake by human. Eigenvectors and eigenvalues of the K matrix to simulate the dynamic behavior of the chemical mass in each compartment and variations in human intake through different exposure pathways. For both chemicals, mass in each compartment reaches its peak at 1.5 hour, at the end of the application phase. Of special interest is the contrast in mass evolutions between high and low volatile chemicals. For benzene the two air compartment tends to have the largest mass of chemical after the products is applied, whereas the mass on the near- and far-person surfaces are also high for 2-butoxyethanol. For both chemicals, inhalation exposure dominates the human intake during application phase. Compared to low volatile chemicals (2-butoxyethanol), dermal uptake is negligible for high volatile chemicals (benzene). The adapted two-zone model and its dynamic version using eigenvalues and eigenvectors enables us to describe well and with parsimony the dynamic of masses and intakes. The presentation further details and contrast the time evolution and the resulting intake fraction and product intake fraction for the 21 considered substances.

MO216

Evaluation of models for gas-particle partitioning of nitro- and oxy-aromatic hydrocarbons

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Gas-particle partitioning is an important mechanism affecting the transport and fate of semi-volatile organic compounds (SOCs). The preferential partitioning of SOCs in the atmosphere depends on parameters such as the compound's molecular structure as well as particulate matter physical and chemical properties. This can be explained by various empirical and theoretical models based on single- or poly-parameter linear free energy relationship (ppLFER). To explain the SOC partitioning, each model considers one or more of the compound's physico-chemical properties or particulate matter characteristics. Despite the past efforts in determining the most appropriate model, discrepancies remain between the model prediction and observation because some models neglect certain intermolecular interactions. The aim of the present research was to apply a dual-phase as well as a multi-phase ppLFER model to predict gas-particle partitioning of nitro- and oxy- aromatic hydrocarbons. To this end, air samples (gas and particulate phase) were collected from urban and non-urban sites in Germany, France, and India. The poster will present the model predictions versus observations, and discuss their structural differences in details.

MO217

SimpleBox4nano, A Screening level Environmental Exposure Model for Nanoparticles: Analysis of TiO2, Ag and C60

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In order to sustain the development of nanomaterials by industry, a robust risk assessment framework is needed. A screening level environmental exposure model is one of the first steps of such a framework currently in place in Europe and the United Sates, but this needs adaptation for use with nanomaterials. We incorporated the SimpleBox4nano model definition previously published into the Simplebox model currently implemented in EUSES as part of REACH. This is a nested multi-compartment multi-scale model that can predict exposure concentrations (PECs) for 4 different chemical species in the environment. We use this model to show the uncertainty of estimating PECs for TiO2, Ag and C60 nanoparticles (nPs). These nPs represent three common nP types, being a poorly soluble metal oxide, a readily soluble metal and an insoluble carbonaceous nP, respectively. The default scenario of SimpleBox v4 is used, which includes the Rhine catchment, Europe and the whole Northern hemisphere. In order to assess the uncertainty of the steady state PECs we conducted a probabilistic assessment based on the uncertainty in nano specific input parameters. The variability and uncertainty of other parameters was not included due to this being similar for conventional chemicals and is expected to currently be at an acceptable level for screening purposes. The results show that the variation of the obtained PECs can be explained largely by the uncertainty in the emission rates. Other important parameters explaining the variation in PECs are (i) the uncertainty in the attachment efficiency between nPs and natural colloids and other particulate matter. And (ii) the dissolution rate of nPs. It was found that the dissolved species of nPs reached very high concentrations in certain environmental compartments, this is however due to the low removal rates from these compartments. The concentrations were much lower when considering the 1 year PECs calculated using the dynamic module of SimpleBox4nano. This suggests that for regulatory purposes, time dependant PECs might be more relevant than steady state concentrations in exposure assessment of nPs.

MO218

A risk management model for evaluating the impact of sediment resuspension on the distribution, bioavailability and toxicity of harmful contaminants in the port of Antwerp and Scheldt Estuary

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The port of Antwerp is Europe's most central and second largest sea port, ranking second behind Rotterdam by total freight shipped. It is a cluster of industry and marine and road traffic in a densely populated area. Present and historical activities have caused the contamination of sediment and water and led to a moderate to low water quality within the harbour docks. Under undisturbed circumstances the impact on water quality of contaminants fixed in the sediment layer is relatively low, but has been investigated to increase significantly when contaminated sediments are resuspended. In order to maintain or improve the current water quality of the Port of Antwerp as well as the connected Scheldt estuary areas, the aim of the present project, which is part of EcoDocks+, a cooperation between the Antwerp Port Authority and the University of Antwerp, is to develop a dynamic risk management model for contaminants under disturbance in the aquatic environment. With this model the main sources for sediment resuspension within the port area will be determined and the re-accumulation behaviour of contaminants in the water phase investigated. It is expected that sediment resuspension and transport and therefore the distribution, bioavailability and toxicity of harmful contaminants is mainly caused by three major factors (1) advective sediment flux through the sluices, (2) ship traffic and (3) dredging activities. In the coming four months, existing literature and data sets as well as the results from own measurements will be used to develop a user friendly interface that can be used to calculate the short and long term changes in concentrations of contaminants in water and sediment layers during and after resuspension and to determine the effect size on the aquatic environment of each of the three factors. In the future, our results and the model itself can be used to identify areas with high risk potential and evaluate possible risks of future maintenance or construction works on harbour sediments on current water quality status.

MO219

Does the inclusion of mass balance and degradation processes to vegetative filter strips impact long-term pesticide environmental exposure assessments?

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Vegetative filter strips (VFS) are a widely adopted for reducing pesticide transport from adjacent fields to receiving water bodies. The long-term VFS efficacy is dependent on site-specific factors related to soils, weather, land use, vegetation and maintenance. The previous version of the well-tested process-based model for VFS (VFSMOD), assumed that pesticide mass stored in the VFS was not available for transport in subsequent storm events. This research study uses an updated PRZM/VFSMOD/EXAMS modeling framework, by considering the effect of the addition of surface mass balance and four options of degradation processes on acute (peak) and chronic (60-d) aquatic environmental exposure concentrations (EECs) and percent reductions across three distinct 30-yr US EPA scenarios. Global sensitivity analysis (GSA) was used to assess the relative importance of adding or removing mass balance and degradation processes in the context of other important input factors like VFS length (VL), pesticide organic-carbon sorption coefficient (Koc), and half-lives in both water and soil phases. It was concluded that considering degradation in the VFS was not relatively important if single, large events were controlling the transport process, as is typical for the higher percentiles considered in standard exposure assessments. Degradation processes become more important when considering percent reductions in acute or chronic EECs, especially under scenarios with lower pesticide losses.

MO220

Development of a scoring system for surface soil pollution potential of chemicals from accidental release by using a multimedia model SoilCCA

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Hazardous chemicals accidentally released into air can transfer to surface soil by dry and wet depositions, leading to surface soil pollution which can adversely impact the soil ecosystem. A new scoring system was developed to evaluate the surface soil pollution potential of chemicals accidentally released into air. Scores for surface soil pollution potential of individual chemicals were to be determined by multiplying three indicators i.e., level of pollution, persistence of pollution, and spatial extent of pollution given a unit quntity (1 ton) of release. A new dynamic mutimedia model named SoilCCA was developed to calculate the scores. SoilCCA consists of a toal of 3100 cells to cover an area of 15 km (L) x 3 km (W) as a model domain. Each cell is of a 150m x 150m x 1000m size with three comparments, i.e., air, surface soil, and vegetation. The temporal and spatial concentration change in surface soil was investigated for five typical types of chemical release. It was found that the concentration profiles with time and space were practically independent of the release type with a difference only in the concentration level and the rate of decrease of the concentration among the chemicals. From the findings, the three equations were derived to calculate the indicators; i) pollution level as represented by the average concentration, ii) persistence of pollution by the slope of linear relationship between logarithm of the surface soil concentration and time, and iii) spatial extent of pollution by logarithm of the maximum concentration in surfac soil. About 600 chemicals in the list of "Toxic Chemicals Control Act" of Republic of Korea will be evaluated for their surface soil pollution potential.

MO221

Evaluation of accidentally released chemicals for their surface soil pollution potential by using SoilCCA

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Hazardous chemicals accidentally released into air were evaluated for their surface soil pollution potential. Scores for surface soil pollution potential of individual chemicals were to be determined from three indicators (level of pollution, persistence of pollution, and spatial extent of pollution given a unit quntity (1 ton) of release) by using SoilCCA. About 600 chemicals in the list of "Toxic Chemicals Control Act" of Republic of Korea were evaluated. Heavy metals and semi-volatiles generally showed higher scores than VOCs, which was qualitatively anticipated prior to the evaluation. Chemicals of the greatest surface soil pollution potential include heavy metals and semi-volatile organic compounds such as benzo(a)pyrene as their wet deposition flux and sorption capacity onto soil particles are high while the loss rates by diffusion to air and surface run-off are low. For the organic compounds evaluated, only the indicator for the spatial extent of pollution showed negative correlations with the Henry's law constant exceeding a certain value depending on the chemical groups. The low pollution potential of VOCs can be accounted for primarily by their high vapor pressure and Henry's law constant. Of particular use of SoilCCA was made of evaluating organic compounds that belong to neither semi-volatiles nor VOCs, which would not readily be done without a quantitative tool.

MO222

Monitoring and modelling of POPs in air at Cape Verde, Africa

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Ambient air is among the core media selected for the sampling and analysis of Persistent Organic Pollutants (POPs) under the Stockholm Convention. Ambient air also provide useful information for (i) studies of global transports of POPs, and (ii) atmospheric sources and source regions. Yet, existing data on POPs in air based on active air sampling remain scarce and mainly limited to industrialized regions in the northern hemisphere. The primary objectives of this study were to (i) monitor concentrations of selected POPs in air outside West Africa, and (ii) to evaluate sources and source regions affecting measured concentrations. For this purpose, an active high-volume air sampler was installed on The Cape Verde Atmospheric Observatory (CVAO: 16.848°N, 24.871°W), a World Meteorological Organization (WMO) global station at Cape Verde outside the coast of West Africa. Sampling commenced in May, 2012 and 43 samples were collected until June 2013 (24 h sampling). The samples were analyzed for selected polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), hexachlorbenzene (HCB) and chlordanes. The source regions of the air masses were evaluated for each sample using the FLEXPART model. FLEXPART is an atmospheric Lagrangian transport model, developed by Andreas Stohl and co-workers, which calculates the trajectories of so-called tracer particles (flexpart.eu). It was run in backward mode, in order to identify the source regions of air pollutants at a particular site. A general finding is that the concentrations measured are in the lower range in comparison to data from the literature, which is mainly attributed to the remoteness of the station from major sources and source regions. Factors controlling measured concentrations will be evaluated and discussed.

MO223

Modelling the behavior of cyclic volatile methyl siloxanes in an Arctic benthopelagic freshwater food web

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Cyclic volatile methyl siloxanes (cVMS) are used in personal care products and are emitted to aquatic environments through wastewater effluents. Due to the high hydrophobicity of cVMS, sediments are expected to be an important source for biotic exposure in benthic and benthopelagic food webs. However, details of the exposure pathways have yet to be fully understood. The main objective of this study was to explore the role of the sediment compartment for bioaccumulation of cVMS in an Arctic lake (Lake Storvannet, 70 °N 23 °E) through a combination of modelling and measurements. Storvannet is an oligotrophic lake with ice-cover for 6 months of the year and a short summer season. Food webs for resident Arctic Char (*Salvelinus Alpinus*) and Brown Trout (*Salmo Trutta*) are strongly connected to the benthic community. Sediment, benthos, and fish were collected and analyzed for cVMS concentrations in 2014. Concentrations of decamethylcyclopentasiloxane (D5) were comparable to concentrations found near more highly populated areas, and were significantly higher in muscle tissue of Arctic Char (3.8 ± 2.8 µg/g lipid) than Brown Trout (0.8 ± 0.6 µg/g lipid). Higher values of $\delta^{15}N$ in Brown Trout (12.4 ± 0.9) than in Arctic Char (10.4 ± 1.0) indicates trophic dilution, but their different cVMS concentrations may also be influenced by species-specific differences in behavior or biotransformation. To rationalize the measured concentrations, a mechanistic pelagic bioaccumulation model (ACC-HUMAN) was modified to incorporate benthic exposure. The model was specifically parameterized for key benthic species (the deposit feeder *Chironomidae* sp. and the filter feeder *Pisidium* sp.) and fish, including time-variant uptake and elimination processes for ventilation, feeding, growth, egestion and biotransformation. Measured concentrations in sediments and modelled concentrations in water were used as input to the model. Preliminary simulations show that incorporation of a benthic link in ACC-HUMAN can be important to rationalize not only observed concentrations, but also cVMS oligomer patterns in fish. The model was used to explore the influence of different dietary scenarios, as well as observed concentration differences between Arctic Char and Brown Trout. This model-measurement comparison helps to both evaluate the new benthic ACC-HUMAN model and to contribute new insights into the trophic transfer of cVMS in benthopelagic ecosystems.

MO224

Critical review and interpretation of environmental data for volatile methylsiloxanes: Monitoring cVMS in non-biological media

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Due to their heavy use, cyclic volatile methylsiloxanes (cVMS) are under increased regulatory and scientific scrutiny. In last few years, the fate, transport and distribution of these compounds have been studied both by multimedia environmental fate modeling and also by the environmental monitoring. The objective of this project was to compare the monitoring results of cVMS with their model predictions and to identify the critical data gaps in our understanding of the major environmental processes regulating the transport and distribution of cVMS in the total environment. The published modeling assessments included both simple steady state simulations and time variable analysis at local, continental and global scales using both hypothetical and realistic release scenarios. The non-biological media studied in the disclosed environmental monitoring programs included air, soil, water and sediment mostly in regions impacted by human activities with some from remote Polar Regions. The temporal and spatial patterns of cVMS concentrations in air, soil and sediment, were generally in a good agreement with the modeling predictions with exception to a recently published findings in Antarctica. The biggest inconsistency with the model predictions was the concentration ratios of various cVMS measured at different locations. This highlights that future work is needed to develop monitoring methods which can prevent samples from contamination and to minimize artifacts and misinterpretation in reporting cVMS environmental behavior including the concentration ratios.

MO225

Temporal-spatial fate of cesium-137 in terrestrial environment around Fukushima Daiichi Nuclear Power Plant: a challenge for daily simulation

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About five years has passed since the accident of the Fukushima Daiichi Nuclear Power Plant (FDNPP). Topic of interest has shifted from analyzing the current situation of radioactive pollution to predicting the future situations of radioactive pollution especially of cesium (Cs)-137. Previously, we reported simulation results of terrestrial environmental fate of Cs-137 in Japan about 10 years after the accident, using the multimedia environmental fate model (Grid-Catchment Integrated Multimedia Modeling System, G-CIEMS). Based on this simulation result and published researches, it was revealed that major part of Cs-137 had deposited to forest area, most of Cs-137 strongly attached to soil surface after the deposition, and therefore Cs-137 would slowly run off from the forest area. Several researchers reported that outflow of Cs-137 to the ocean predominantly occurred during typhoon Roke September 21, 2011. Therefore, in this study, we improved our model in order to simulate daily change of environmental conditions and related fate of Cs-137. To achieve this goal, we 1) calculated amounts of direct deposition of Cs-137 to surface waters (rivers and lakes), 2) calculated daily amount of soil runoff from land area by using Universal Soil Loss Equation (USLE), 3) set up daily river flow rate, and 4) calculated daily suspended solid (SS) concentration in river. As the first step, we made polygon data of surface water segments which were used in our model, calculated the area of each surface water segment in each 1-km mesh, and then calculated amounts of direct deposition of Cs-137 to each surface water segment. As the second step, we calculated daily “rain factor” used in USLE using 30 minutes precipitation data for each 1-km mesh, and then calculated daily soil loss from land by erosion. As the third step, we set up daily flow rate of each river segment based on daily precipitation and simple assumptions. As the fourth step, we calculated daily SS concentration in each river segment using daily input amounts of SS (= soil loss from dry land) and mass balance equation of SS. We simulated annual fate of Cs-137 in Abukuma river basin and river basins in Hamadori region, the surrounding area of the FDNPP, and obtained good agreement between simulated flux of Cs-137 and field observations.

MO226

Long-term and large-scale prediction of air radiation dose rate in Fukushima: incorporating vertical migration of radioactive cesium

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Long-term prediction of air radiation dose rate is important in Fukushima, Japan, where a large amount of radioactive cesium was deposited following the accident of Fukushima Dai-ichi nuclear power plant in March 2011. In general, air dose rate derived from deposited radioactive cesium is known to decline with time, not only because of natural decay, but also because of various processes, including migration of cesium into deeper soil. The rate of decline excluding natural decay would vary depending on locations and other factors. To date, however, there has been no comprehensive study on this. In the present study, we evaluated the effect of radioactive cesium migration on the decline of air dose rate in a large area, using various geo-referenced and time-course data of deposition and soil depth-profile of radioactive cesium and air dose, which have been published since the nuclear accident. The decline of air dose rate was evaluated, after eliminating the effect of natural decay, as the temporal change of conversion coefficients between the depositional radioactive cesium amount and the air dose rate measured at 1 meter above the ground. The soil migration of radioactive cesium was evaluated by the temporal change of weighted center obtained from the depth profile. The results showed that the conversion coefficient decreased with time and with depth, although a large variation was noted on the temporal change. Finally, we predicted the long-term change of air dose rate in the large area of eastern Japan, considering the effect of migration of radioactive cesium on the conversion coefficient. Our study highlights the importance of vertical migration of radioactive cesium for a better spatiotemporal prediction of air dose rate.

MO227

A Global Historical Inventory of Individual PBDEs: From Production Towards Emissions

A. Golnoush, NILU Norwegian Institute for Air Research / Geography; K. Breivik, Norwegian Inst. for Air Research

Despite advances in environmental fate modeling, measuring and monitoring of persistent organic pollutants (POPs), knowledge about their sources and emissions remains a major knowledge gap. This mitigates our potential to understand and predict historical and future POPs concentrations in various environmental media. The first step towards estimating the global emissions of deliberately produced POPs and their future trends is to obtain an overview of their production and consumption on a global scale. In this study, we compiled data on the global production of three main commercial mixtures of PBDEs (penta, octa and decaBDE) from 1970, along with data on the chemical composition of these mixtures, in order to estimate historical production trends of selected individual PBDE congeners. The global consumption over time was next estimated from available information on use of PBDEs in different regions, per capita consumption and/or surrogate data. These results will be coupled with material flow analysis to estimate the amount of waste PBDE-containing products that are subject to transboundary movement. The results of this study will be used in a global emission model to estimate temporal trends of PBDE emissions at the global scale.

MO228
Environmental exposure assessment of sucralose in receiving waters at differing spatial scales
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Down-the-drain exposure models provide a valuable screening-level tool for estimating environmental exposure to product ingredients which are treated and discharged at municipal wastewater treatment plants. We present an environmental exposure assessment for sucralose, an artificial sweetener which ultimately ends up in the environment via down the drain emissions. Exposure modeling was performed using the iSTREEM® model, a publically-available web-based model supported by the American Cleaning Institute (www.istreem.org) which estimates spatially-explicit concentrations of chemicals in effluent and receiving waters across the U.S. at mean and low flow conditions. Wastewater treatment facility influent loadings of sucralose were estimated using per-capita usage derived from market sales volume combined with individual facility population served and daily flow estimates within the iSTREEM® model. The screening-level assessment used an assumption of zero removal during treatment and no in-stream decay, resulting in a representation of "worst-case" environmental exposure estimates. Three case studies of modeling at different spatial extents are presented: national scale of the continental U.S., regional scale of the Lake Erie drainage basin, and local scale of the Grand River Watershed in Canada. US-wide predicted environmental concentrations (PECs) estimated by the model at mean annual flow conditions were comparable to sucralose concentrations typically expected to be observed in the field, with a 90th percentile PEC in surface waters of approximately 1.9 μg/L. Watershed-scale modeling of the Grand River was compared to published data from 23 sites measured in 2007-2009. This local assessment was enhanced with temporally-specific adjustments to flow. Once time-specific gaging data were added, the model predicted a comparable exposure pattern to those measured across the 23 sites. Maps of the estimated geographic distribution of US-wide and Grand River watershed river concentrations are presented using geo-referenced concentration data generated by the iSTREEM® model. These screening-level environmental exposure assessments provide an estimated distribution of PECs in a spatial and potentially temporal context. These can be used to inform risk management and/or subsequent higher-tier assessment.

MO229
FlowEQ - a coupled flow-network and fugacity based fate and transport model for the assessment of Bisphenol A in the environment
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Substances used in consumer products can enter the environment as part of the manufacturing process or through the use and disposal of consumer products. These substances can be discharged through a number of different pathways such as manufacturing outfalls, domestic wastewater, and other emissions pathways. When these substances are discharged to surface water they have the potential to accumulate in aquatic environments depending on their physico-chemical properties. Many of these substances are infrequently characterized in aquatic environments due to inadequate analytical methods or the lack of their inclusion in monitoring programs. Furthermore, even a robust monitoring dataset often provides limited information on the relative importance of source pathways. The first step in the assessment of the potential impacts to the environment is an assessment of the concentrations in environmental media and the relative importance of the various source pathways. Models such as MORE/MONERIS have been developed to quantify emissions and model the resultant surface water concentrations using a flow network approach that utilizes a water balance/dilution methodology. This approach has been shown to provide accurate predictions for certain substances, but it does not model processes such as partitioning between various media and degradation. To address this limitation, we developed a hybrid fate and transport model that combines the dilution/water balance flow network of the MORE/MONERIS model with a level III fugacity based model (*FlowEQ*). Partitioning between media and degradation processes are easily included in fugacity based models. The *FlowEQ* model was populated with watershed information and emissions of Bisphenol A (BPA), a substance that degrades in the environment, to German watersheds. The model considered a wide variety of processes including industrial and per capita emissions, wastewater treatment, landfill leachate discharges, partitioning between environmental media, soil runoff, atmospheric scavenging, degradation, and the advective transport to downstream areas. Consumer emissions were found to dominate and the predicted surface water concentrations were consistent with those measured in surface water demonstrating the suitability of the *FlowEQ* model. The model can be further used to assess the relative importance of various substances and emissions pathways and explore how concentrations change as usage patterns and treatment technologies change.

Modelling of pesticides and biocides fate and exposure in a regulatory context (P)

MO230
Modeling honey bee extinction risk posed by neonicotinoid insecticide (imidacloprid) exposure
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Colony collapse disorder has become an ecology crisis for honey bee population in recent years. Neonicotinoid insecticide is the suspected risk factor, which may accelerate the bee population decline. The most widely used insecticide in the world is imidacloprid that can harm honey bees through their pollinating of nectar and other consumes. The purpose of this study was to develop a risk-based population dynamic model and a probabilistic risk assessment framework to predict the potential hazards of imidacloprid toxicity toward honey bee population that may further casue colony collapse disorder.Published toxicity bioassay of imidacloprid exposure to honey bee mortality were be used as the study data. The nollinear regression models were used to reconstruct a dose-mortality response profiles in acute and chronic imidacloprid toxicity. We rebuilt the simple differential equation-based model built on previous study that can be used to simulate the seasonal dynamic of honey bee population and food storage based on the seasonality parameters of laying rate, mortality rate, and food consumption rate. This study also parameterized the imidacloprid toxocity to simulate the toxicity effect on honey bee population. Finally, we applied the probabilistic risk assessment framworktoassess the potential risk of environmentally relevant imidacloprid for hooney bee population. We further built the interactive web application by using Shiny for the R programming language that have the potential to revolutionize the sharing and risk visualization of population dynamic model simulations. This study provides the novel risk assessment concept to characterize the imidacloprid exposure risk to honey bee population. Current result showed that realisitc in-field exposure dose distribution of imidacloprid can only effect the honey bee population slightly. Moreover, the developed population dynamic model can be further extended to simulate and realize the other risk factors that may cause colony collapse disorder. The web-based interactive applications of risk visualization can help people understand the quantitative risk of imidacloprid toxicity.

MO231
Modelling of Acetamiprid residues as foliar application in pepper
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Pesticides are worldwide used to control pests, diseases and weeds of many crops. Although pesticide residues are regularly monitored and regulated, they are still detected above MRL (maximum residue level) in many countries. Many models have been developed to predict the residues in plants and crops for soil application and are not consider foliar application for the following pesticide application. Acetamiprid is a systemic insecticide applied to pepper plants twice in ten days intervals and collected pepper fruits 35 days long. Quechers method was used for extraction of acetamiprid and analysed by LC/MS/MS. The cascade model was adopted to foliar application to predict the pesticide residue over the period. The concentration of acetamiprid residues declined very sharply until second application. Simulated concentrations of residues were very similar to experimental results so that curve fitted well. The concentration in pepper reaches almost fifty percent of initial concentration after second application and decreased slowly in comparasion to first application. The calculated concentrations in fruit after second application were lower than experimental. This model has advantage to predict PHI (Preharvest Intervals) between applications to decrease acetamiprid residues in many crops.

MO232
Computer simulations of plant protection products on leaf surfaces at the nanoscale

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Foliar applied plant protection products (PPPs) may be washed-off the leaf surface during rainfall events, leading to varying temporal exposure patterns of the pesticides on soil. Good descriptors of this process are, however, still missing due to a lack of understanding of the key factors involved. Thus, current environmental fate regulatory assessments for PPPs rely on worst case descriptors to account for the wash-off process. The objective of this work is to use molecular modeling to fill in the gap and allow for the establishment of higher tier refinements for environmental fate models. Using molecular dynamics simulations, we can elucidate the relevant interactions involved at the nano/micro-scale using coarse-grained models of the PPP's formulation components (active ingredients, dispersing agents, emulsifiers, solvents, etc.) and of the plant leaf surface. The leaf surface is simulated by a thick hydrophobic layer covered by a mixed polymer matrix of the main epicuticular waxes. The composition of this mix can be obtained from ATR-FTIR spectroscopy measurements for the specific plant of interest and thus characterizes the modeled interactions for the various types of crops under investigation. All coarse-grained models are based on the individual molecules' octanol-water partitioning. By examining how the individual formulation components are distributed on the leaf surface with respect to the aqueous phase of the PPP, we aim to estimate its wash-off potential.

MO233
Experimental determination of foliar wash-off factors as input for FOCUS exposure modelling
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The foliar wash-off factor is a compound and product (formulation) specific modelling input parameter relevant for the aquatic exposure assessment after foliar application. To ensure a realistic worse case for soil residues, the foliar wash-off coefficient was originally set to a default value of 0.5 cm^{-1} for FOCUSsw calculations. EFSA proposed later a more conservative default value of 1 cm^{-1} if no experimental data are available. The use of measured refinement values for the wash-off fraction reflects specific compound behaviour and is part of FOCUS guidance and accepted by EFSA. Recently EFSA published a guidance document in which the foliar wash-off factor also needs to be considered for the soil exposure assessment. Thus, the foliar wash-off factor may be experimentally determined if a refinement is needed for the aquatic or soil exposure assessment of a specific compound. A generic frame work of an experimental study design has been derived in a workshop organised by the European Crop Protection Association (ECPA). This frame work was used for the experimental determination of wash-off factors for modelling purposes. The wash-off studies were conducted under GLP for different compounds of foliar applied products applying different rainfall scenarios. Four main conclusions could be derived from the presented measurements: The wash-off factor … → … is independent from the investigated crop and BBCH stage, if leaf surface properties are comparable regarding their respective wetting regimes. → … can be influenced by both the formulation composition and the physico-chemical characteristics of the active ingredient, in some cases strongly. → … is comparable for standard, non-optimized formulations where the nature of the deposits is similar (e.g. SC, WP and WG formulations). → … of optimized formulations (resistance to wash-off by rain) can be significantly reduced compared to standard formulations.

MO234
Exposure and Risk Assessment for operator exposure to Insecticide thiamethoxam during grape cultivation in vineyard using Whole Body Dosimetry
J. Lee, E. Kim, Seoul National University / Department of Agricultural Biotechnology; Y. Shin, Seoul National University; J. Lee, J. Lee, M. Jung, J. Sung, Seoul National University / Department of Agricultural Biotechnology; J. Kim, Seoul National University
Thiamethoxam belongs to the neonicoitinoid class of chemicals. It is broad-spectrum insecticides that are used to control a wide range of diseases in fruit and vegetables. Assessment for operator's dermal and inhalation exposure to thiamethoxam during cultivation of grape in vineyard was carried out. For dermal exposure measurement, whole body dosimetry (WBD) was performed in Korea. WBD consists of cotton/polyester outer clothes and cotton inner clothes. Hand exposure was measured by washing of nitrile gloves and hands while head exposure was monitored by face/neck wipe technique. Inhalation exposure was monitored with personal air sampling pumps and IOM sampler (glass fiber filter). Analytical limit of quantitation was 5.0 ng/mL. Good reproducibility (C.V < 7.6%), linearity ($R^2 > 0.99$) and recovery (75~113%) were obtained. Field recovery of thiamethoxam was 88~106%. Field experiments were carried out 11 replicates, and data was processed with 75 percentile statistical method. During application, total dermal exposure was 32277.0 µg, and that of mixing/loading case was 194.5 µg. During mixing/loading, hand exposure of thiamethoxam (156.4 µg) was slightly less than that of application case (519.3 µg). But considering the ratio of hand exposure to the total dermal exposure, mixing/loading hand exposure ratio (80.4%) was much higher than that of application (1.6%). During mixing/loading, all parts were similar density, but application case, exposure of thigh and shin was high density (47.0%). Penetration rate between outer and inner dosimeter was about 10%. Inhalation exposure during application (10.8 µg) was about 30 times more than that of mixing/loading case (0.3 µg). Margin of safety (MOS) was calculated for risk assessment using male Korean average body weight (70 kg) and acceptable operator exposure level (500 µg/kg/day) to give 7.3, suggesting that health risk of operator during treatment of thiamethoxam for grape in vineyard could be safe.

MO235
World Exposure Assessment Tools and Scenarios
A. Ritter, Waterborne Environmental, Inc.; M. Cheplick, Waterborne Environment; G. Hoogeweg, C.M. Holmes, Waterborne Environmental, Inc.
Modeling platforms have been developed to evaluate the potential impact of crop protection chemicals on the environment throughout the world. The tools currently have been configured with scenarios containing crop, soil, and weather conditions for major agricultural areas in Brazil, Canada, Colombia, the European Union, Norway, the People's Republic of China, and the United States. Additionally, agricultural scenarios have been developed for countries such as Peru, Ecuador, Taiwan, Philippines, and Korea. All these scenarios are simulated using fate and transport models that have been accepted for regulatory assessment in the U.S. and the European Union, including the Pesticide Root Zone Model (PRZM), Exposure Analysis Modeling System (EXAMS), Rice Water Quality Model (RICEWQ), and Toxic Substances in Surface Waters (TOXWA). Development of country specific scenarios and tools will be described. A key strength of the tools are that scenarios can be added for additional geographical areas with relative ease and the appropriate regulatory endpoints.

MO236
First industry experiences with the new exposure assessment guidance documents and models in China
H. Shbaita,
Since almost a decade ago, China has started to develop their own framework for the registration of plant protection products. A major building block of this framework is the guidance documents on environmental risk assessments. In total there are 6 (draft) guidance documents, aquatic (rice), groundwater, birds, bees, NTA and silkworms. These include two models for groundwater calculation (ChinaPEARL) and one for surface water paddy rice (TopRice). After the first commenting round in beginning of this year, in which CLC (CropLife China), the CERA team (Chinese Environmental Risk assessment) and CLI (CropLife International) provided more than 400 comments, the guidance documents have been updated and are now in the process of finalization (most probably, by the time the SETAC conference takes place, these guidance documents will be already published and came into force). ICAMA has given training using the final draft guidance documents to different stakeholder. This gives the ideal time point to report on the first experiences the industry has made using these "new" exposure assessment models and guidance documents. This includes comparison between EU and China PEARL, and also model testing for the TopRice model.

MO237
Modelling and monitoring travel distance of pendimethalin in air
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Based on the physical-chemical properties of pendimethalin volatilization can be a relevant dissipation pathway. Laboratory studies with pendimethalin SC and EC formulations showed significant volatilization from plant and soil surfaces. The travel distance in air is mainly influenced by the aerial half-life of pendimethalin which is estimated to be 4.2 hours (Atkinson calculations) and confirmed by experimental studies. Multimedia models were used to estimate the distribution and expected concentrations in environ-mental compartments at different distances from the source. Aerial transport parameters such as characteristic travel distances (CTD) were also derived. Existing monitoring data show that pendimethalin findings are highly correlated with time and location of application. The modelling results confirm that aerial transport of pendimethalin is possible over a few tens of kilometres whereas transport over longer distances to remote areas is unlikely. Mitigation of aerial transport is achieved with a new encapsulated CS formulation. A significant reduction of the volatilization rate of encapsulated pendimethalin compared with EC and SC formulations was shown in laboratory and wind tunnel (semi field) experiments where also the transport and deposition of pendimethalin was significantly reduced. Trace detections of pendimethalin in air can be further minimised by use of appropriate application techniques and avoiding application at unfavourable climatic conditions. Adjuvants that reduce the volatility of pendimethalin containing products are under evaluation.

MO238
A moni-modelling approach for risk management of pesticide at territorial level
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In general, approaches used by water quality managers to preserve water bodies

from the risks associated to the use of Plant Protection Products (PPPs) fall within two categories: monitoring campaigns or predictive models. Both approaches show pro and cons. Monitoring programs are required in current EU regulations, as they are very useful to verify whether the concentration of chemicals exceed predetermined trigger values (i.e. for PPPs it is 0.1 µg/L in groundwater). On the other hand, they represent a single point in space and time (static), in a situation in which different dynamic processes act at the same time; consequently, they cannot be used to forecast the future state of the environment. On the other hand, predictive models are very useful to spatially represent the forecasted contamination starting from several sources on the territory. However, they rely on assumptions, mainly related to the parameterisation of the model inputs which could introduce biases and uncertainties in the spatial estimation of pesticide transport toward water resources. In our opinion, a new methodology that provides the integration of both approaches (what we here call moni-modelling approach) could provide invaluable help for risk managers. In fact, this approach would be very useful, particularly on a territorial scale, for a proper risk management of PPPs. In fact, it would be helpful to: a) identify sensible areas where implement mitigation measures or limitation of use, b) re-design future monitoring plans, c) better calibration of the pedo-climatic input data for the environmental fate models. As a case study, the proposed approach has been applied to Lombardy region (a NUTS 2 zone in North of Italy) using six active ingredients with different leaching behaviours.

MO239

The Wash-off Model - A new approach to estimate wash-off of biocides from different surfaces

B. Kind, WSC Scientific GmbH

Biocidal products can be applied outdoors on various surfaces like terraces, balconies, walls, fences, pavements etc. as a protection against insects or different herbs. These products can then be washed off by precipitation and reach soils and drainpipes. This route of entrance needs to be considered in the Environmental Risk Assessment of Biocides. Unfortunately, the proportion of the active substance washed off is rarely known so that complete wash-off immediately after application is often assumed as a worst-case. Since this is rather unrealistic, a wash-off model was developed for different surfaces, which calculates the amount washed off due to a known wash-off factor which has been the proposed procedure so far. Additionally, the amount can be estimated using the substance water solubility and the mean precipitation. The amount being washed-off may further be reduced by losses due to photodegradation and volatilization between the application and the first rainfall event. Relevant input parameters are the amount of active substance applied (mg/m^2 or mg/m), the area (m^2) or perimeter (m) of the outdoor surface based on the Emission Scenario Documents (ESDs) and, if possible, the volatilization rate (d^{-1}) and photodegradation half life (d) along with the time (d) between application and the first rainfall. If the last three parameters are not known, default values of 1000 days for photodegradation, 0 for volatilization rate and 0 days between application and rain are assumed as worst-case. For the established approach only the wash-off factor has to be entered in addition to the inputs mentioned above. For the alternative approach, the user has to enter the water solubility (mg/L) along with a mean rainfall amount (mm). Data on precipitation can be estimated using e.g. FOCUS scenarios from the current FOCUS models (e.g. FOCUS-PEARL 4.4.4, FOCUS-PELMO 5.5.3). The two approaches are demonstrated in a short example. A substance is applied with the amount of 10 mg/m^2 on a 30 m^2 area, resulting in 300 mg a.s. applied and washed off when using a worst-case wash-off factor of 1. But due to its low solubility of 1 mg/L and assuming a mean rainfall of 4 mm, the actual amount washed off during the next rain event is reduced to 120 mg a.s. In conclusion, the wash-off model can help to estimate realistic wash-off amounts of biocidal products from outdoor surfaces, either based on a given wash-off factor or on the water solubility of the active substance.

MO240

Outdoor use of Biocidal products: considering contaminated rainwater pathways in the urban context and potential for harmonisation

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Contaminated rainwater and, in particular, pathways of exposure to surface water via sewage treatment plant bypass and separated rainwater systems, are increasingly considered within Biocidal risk assessments. A first tier approach was designed for outdoor application of a number of preservative products (UBA, 2014) and there is indication that other product types may be considered in the future. In addition, an existing higher tier approach involving the HardSpec model is commonly used for plant protection products applied in urban areas and it is proposed that the data generated for the design of this model could be the basis for a second tier approach for biocides. Particular consideration is given to those substances exhibiting a strong potential for dissipation and to those uses where infiltration and retention may be significant.

MO241

In silico Prediction of the Formation of Non-Extractable Residues (NER) in Soil with Regard to their Environmental Hazard

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Organo-clay complexes are the major sink for xenobiotics in soils and sediments. Pollutants can be degraded and transformed by biotic and abiotic mechanisms. Additionally, the parent xenobiotics and their transformation products are immobilized as non-extractable residues (NER). All chemicals are assumed to form NER to different extents, but the actual amount of NER formation depends not only on the chemical structure but also on the environmental conditions. Three types of NER have recently been classified: Type I are xenobiotics sorbed or entrapped, type II are xenobiotics covalently bound, and type III are biogenic residues. The possible remobilisation of incorporated xenobiotics is of environmental concern and refers particularly to type I NER. Type II or type III NER formation is considered not to cause harm. We therefore launched a project aiming at predicting the potential for formation of different types of both xenobiotic and biogenic NER formation from xenobiotics, in particular pesticides. To this end, derivation of respective structural alerts had been envisaged originally. However, we found that the available experimental data are not sufficient to derive general structural alerts. Structural alerts thus can only address a subset of potential NER forming chemicals. Acknowledging this restriction, the strategy has been altered and now focuses on identifying appropriate thresholds of physicochemical and molecular properties and their control on NER formation. The paper provides an overview of the original and adapted approach and reports modelling results. The financial support of this study by CEFI-LRI (ECO24-UFZ) is gratefully acknowledged.

MO242

Kinetic Evaluation of Field Dissipation Studies: Applying MCMC simulator DREAM for Parameter Inference and Uncertainty Analysis

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In legacy field dissipation studies, plant protection products (PPP) were applied to soil surface or to cropped plots. Consequently, surface processes other than (microbial) degradation might contribute to the overall dissipation of PPP mass in a soil profile. The properties of the compound as well as agro-climatic conditions will influence degradation, plant uptake and leaching below the lowest sampling depth, particularly if the depth of sampling is limited. A recent EFSA guidance document [1] provides criteria to be followed to determine a true DegT50 from those field trials. The guidance is lacking some information about the detailed procedure to derive DegT50 of metabolites, particularly if global solvers or inverse modelling approaches are to be considered. This presentation describes a novel methology to derive the true DegT50 of a primary metabolite from legacy field studies employing a global solver. The evaluation considers requirements of latest regulations, i.e. ignoring masses before 10 mm of rainfall, and using state of the art weather data. For an improved understanding of the results of field dissipation studies the leaching model PEARL 4.4.4 was coupled with a Markov Chain Monte Carlo sampler (Differential Evolution Adaptive Metropolis algorithm, DREAM_ZS)[2] to efficiently search the parameter space of the advective-dispersion model. Standard statistical measures according to FOCUS are given, e.g. Chi-square test and an analogon of t-test statistics. This overall procedure allowed for a representation of the distinct processes occurring under field conditions, i.e. degradation, leaching, plant uptake (in cases of cropped trials). Consequently, in legacy studies a true DegT50 can be estimated other than an overall DT50, employing a standard time-step normalisation procedure. We will present the results of field trial experiments along with an analysis of parameter uncertainty. [1] EFSA (2014): *EFSA Guidance Document for evaluating laboratory and field dissipation studies to obtain DegT50 values of active substances of plant protection products and transformation products of these active substances in soil*.European Food Safety Authority. EFSA Journal 2014:12 (5):3662. [2] Vrugt (2009): J. Vrugt, C. ter Braak, C. Diks, B. Robinson, J. Hyman, and D. Higdon. *Accelerating markov chain monte carlo simulation by differential evolution with self-adaptive randomized subspace sampling*. Int. J. Nonlinear Sci., 10:273–290, 2009.

MO243

Estimation of degradation rates of pesticides in surface water from higher-tier effect cosm studies by inverse modelling with TOXSWA

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In water bodies with low flow velocities or with multiple spray drift deposition events, exposure concentrations of pesticides are influenced by their degradation rate in water. $DegT_{50,water}$ values derived from water-sediment studies do often not represent well degradation rates in water in realistic, outdoor cosm systems. Therefore, we developed a method to estimate degradation rates in water from available higher-tier effect cosm studies (which have often limited fate data).Results obtained with this method can be useful in higher tier exposure assessment for authorization purposes of pesticides on a national and zonal level. We derived $DegT_{50,water}$ values from outdoor cosm studies for three compounds with relatively low K_{oc} values (metribuzin, linuron and imidacloprid). In total eight outdoor studies were analysed. Studies were only included if aqueous concentrations were available for at least five sampling times and if the water depth was reported. Concentrations in the sediment were not used for the fit (available for only one of the studies). We minimized the difference between simulated and measured aqueous concentrations using PEST vs 13.0 and running FOCUS_TOXSWA_4.4.2 many times. The quality of the fits was assessed visually (considering also trends in residuals) and by calculating the error level of the χ^2 test as recommended by FOCUS Degradation Kinetics. All fits were found to be visually acceptable. The χ^2 error of the eight studies ranged from 7 to 20% and was on average 11%. The 95th confidence interval of the $DegT_{50,water}$ ranged typically from 75 to 125% of the fitted $DegT_{50,water}$ value. Thus, the estimation procedure resulted in sufficiently accurate $DegT_{50,water}$ values for all eight outdoor higher-tier effect cosm studies. For all eight studies, the $DegT_{50,water}$ values obtained appeared to be shorter than those derived from hydrolysis and water-sediment studies (the difference was typically an order of magnitude and at least a factor of two). \n \n

MO244
VFSmod - A comparison with field derived values of pesticide removal efficiency under controlled conditions
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Consideration of the pesticide removal efficiency of vegetative filter strips (VFS) within the regulatory process has been through the use of standardised values for buffer strips of set classes of width (10-12m and 18-20m) and adsorption (aqueous and sediment associated). These are applied to all compounds for all FOCUS scenarios, all crops and all usage periods. The adoption and usage of more dynamic models, such as VFSmod, by Member State regulatory authorities has been relatively slow, despite efforts to underpin this model with a wide database of field data, improve model parametrisation, and further align scenario development with existing FOCUS frameworks. As a complimentary approach to the use of VSFmod, and in support of a number of regulatory submissions, a series of replicated field studies have been carried out as part of the data package where the efficiency of VFS for specific compounds and specific VFS widths has been tested under controlled conditions representative of a selected worst case FOCUSsw scenario covering the intended use profile. This study explores these FOCUSsw scenario related experimental VFS data with a view to examining VFSmod performance further and potentially expanding the validation dataset that underpins it.

MO245
Spray Drift: Representation, Mitigation and Future Directions
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Spray drift measurements are used to assess the applied spray volume carried downwind from application and deposited on adjacent surfaces. Evaluation reference conditions are detailed in ISO 22866 but allow some variation in environmental characteristics at point of application (wind speed, temperature, humidity), application settings (nozzle height, travel speed), crop and landscape influences (bare ground / grass *versus* crop, choice of crop) and measurement standards (choice of collector, relationship of measurement to edge of field / end nozzle location). As a consequence, drift trials may provide variable drift representations. SETAC MAGPIE WG concluded that consideration of harmonization of testing standards beyond ISO 22866 would be better addressed independently *via* spray physics expert working groups. To improve understanding of spray drift and the regulatory basis for risk assessment, a SETAC workshop (DRAW) is underway to facilitate efforts in a number of areas. - Assemble a database of existing spray drift trials for boom sprayers - Analyse and interpret available data; highlight areas where protocol-derived differences warrant further targeted trial investigations - Develop targeted trials to quantify differences emerging from measurement methodology and choices - Develop proposals for standardized measurements to investigate key drivers or influences on drift - Support further interpretation of existing spray drift trials - Determine which collector type best represents the actual deposition capture by the relevant risk assessment compartment - Develop mathematical modelling as a higher-tier basis for risk assessment refinement The valuable heritage of research on spray drift must be harnessed for the longer-term goal of facilitating greater flexibility with drift representation through mathematical modelling of drift arising from boom applications. As representation of spray drift affects a range of risk assessments (aquatic, non-target arthropods/terrestrial plants *etc*.), the workshop includes consideration of implications for these assessments. A specific work group has been developed to provide European regulatory authorities with an expanded, secure toolbox of risk mitigation measures. This action will enable efficient transition to a new framework of drift representation coupled with options to accommodate change.

MO246
Research of boscalid and markers of exposure to boscalid in honey bees
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The intensification of agriculture has been characterized in last decades by a significant use of pesticides. To protect bees against their effects, it is necessary to develop analytical tools for the detection of these compounds in bee products and in bees. The search for pesticides in bee generally involves native target substances. However they may have been degraded or metabolized by bees or due to delayed mortality findings; thus the parent compound may no longer be detectable in the bee. Evidence of contact between bee and pesticide may, nevertheless, be obtained by detecting metabolites/markers of exposure. However, the study of metabolites and of their toxicity, in the bee is still undeveloped. The reasons are firstly, the lack of information on the nature of the metabolites, and secondly the lack of analytical standards, which are essential for conducting the tests. Our work is part of this context and aims firstly at characterizing metabolites of boscalid in bees, and next to search them into bees from symptomatic colonies. Boscalid was chosen for this study as it is a new-generation fungicide which use is increasing, it has a broad spectrum of activity, and it has been detected in bee matrices. Honeybees were sprayed with boscalid in a Potter spray tower and left in crates 24 h at 28°C before being frozen to stop metabolism. Boscalid and its metabolites were extracted from bees by QuEChERS method prior to analysis by liquid chromatography coupled to high resolution mass spectrometry (LC-Q-TOF). Metabolites research was performed by comparing the retention time and mass/charge ratio between sprayed bees and control ones. Discriminant masses (exact mass, isotopic profile, mass fragments) and empirical formula next obtained were compared with databases and/or in silico fragmentation. Thus 5 markers of exposure to boscalid were determined. Then they were synthesized and quantified in bee samples collected from various beehives located in France. The analysis of boscalid and the 5 identified markers of exposure was performed on 42 bee samples from symptomatic colonies. Among these markers, 4 were present in the samples (levels comprised between 0.2 and 36.3 ng/g). About half samples were positive for at least one of the 6 target molecules whereas boscalid was detected in 11 samples but quantified in only 4 with levels up to 330 ng/g. These results highlight the importance of not limiting the analysis of the parent drug in sentinel organisms such as bees.

Prospective and retrospective soil risk assessment of chemical stressors (P)

MO247
How to deal with different soil organic matter contents in ecotoxicological studies for the risk assessment of soil organisms?
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The risk assessment for non-target soil meso- and macroorganisms is based on ecotoxicological tests performed in standardized test soils as a first step. Depending on the test organisms, the guideline recommendations regarding the soil organic matter content (OM) of the test soil vary. For earthworms, the OECD Guidelines 207 and 222 recommend 10% OM (as peat) for the composition of the artificial test soil. The OECD Guidelines 226 and 232, targeting chronic effects on mites and collembolans, propose a soil OM (peat) content of 5%. The quantity and quality of OM in soils might exert a strong effect on the sorption and bioavailability of tested substances and therefore alter the determined toxicity of the tested compound. Amount and quality of OM used in tests with artificial soil might strongly diverge from the humic materials in natural agricultural soils. EFSA (2012) reports e.g. for the Central Zone of Europe OM contents between 1.8 and 8.6% (10th and 90th percentiles of the distribution), respectively. This adds to the uncertainties accompanying the extrapolation of toxicity endpoints measured under standard laboratory test conditions to the situation in the field. To account for the effects of OM content on sorption and thereby bioavailability of tested compounds, the current Guidance document (GD) on Terrestrial Ecotoxicology SANCO/10329/2002 (European Commission 2002) recommends a reduction factor of two for ecotoxicological endpoints derived from studies with lipophilic substances (defined by a $\log K_{ow} > 2$) in artificial soils with 10% OM. At the time the current GD was established, tests with soil organisms were conducted with 10% OM as peat in the soils exclusively. The OECD guidelines 226 and 232 were adopted later, and therefore no proposal exists in the current GD, how to deal with toxicity endpoints for soil organisms derived from tests with 5% OM. Currently, the risk assessment scheme for soil organisms deals similarly with

endpoints conducted in test soils with different peat contents. This contribution presents the outcome of a comprehensive analysis of all available test data with soil organisms in artificial soils of different OM contents that were submitted to authorities in the framework of PPP registration or active substance evaluation. Moreover, it reviews available literature on compound toxicity for soil organisms in soils of different properties, with a focus on the effects of different soil OM contents.

MO248

Soil enzyme activities reveal re-functionalization of trace metal agricultural contaminated soils after Miscanthus giganteus plantation

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Context The use of contaminated soils for non-alimentary cultures is gaining importance in view of the scarcity of agricultural surfaces and the presence of some potential harmful pollutants in surface horizons that could impact the food chain. But in the case of trace metal contaminated soils few information are available on the effects of land use and soil management changes on soil quality parameters and/or soil health. Suggestion was made in literature that enzymatic activities could be good indictors of soil functioning, providing special attention to confounding effects. In this work the objective was to assess the impact of change of cultural practices on the soil quality, as seen by soil enzyme activities. **Materials and methods** We used trial essays were contaminated parcels were divided in two, one part was left under annual culture and the other was planted with the high biomass plant *Miscanthus giganteus*. The enzymatic activities of the C and N cycle were investigated with a diachronic follow-up of β-glucosidase and urease activities, quantified on fresh soil triplicates using colorimetric methods and microplate assay. The effect of miscanthus plantation was also followed up in a soil profile. Soil samples were collected in spring from 2009 to 2011 at 0-10 cm in a trace metal contaminated site with an organic origin of pollution. Sampling in the soil profile was done every 10 cm in depth until 50 cm. **Results** Results for the diachronic follow up in surface horizons showed that soil urease activities under miscanthus differ clearly from those under annual culture with higher values under perennial culture. This was particularly observed as the setting-up of miscanthus was former. β-Glucosidase activities, while less indicative, also suggested an enhancement of microbial activities under miscanthus. When normalizing with the enzymatic activities of the parcel under annual culture, results clearly show the effect of the plantation duration on the re-functionalization of the soil. But despite the rapid answer of the indicators, three years were necessary to significantly quantify an evolution. N cycle was more sensible to point out the re-functionalization of the contaminated soil. In the soil profile results of the enzymatic activities under miscanthus highlighted the evolutionary structuration of microorganisms in contaminated soil. This evolution was faster near the soil surface than in subsurface horizons and better assessed for N cycle.

MO249

Gaining a better understanding of toxic effects on soil communities, using the example of AgNP

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Resource limitations and ethical concerns often hinder field studies in ecotoxicology. One way to meet the frequent calls within the scientific community to increase ecological realism of ecotoxicological test systems is the use of test systems at an intermediate ecological organizational level; this was applied in the present study. Silver nanoparticles (AgNP) were used as an exemplary test system because of their expected accumulation in soils. Ecotoxicological information on this emerging technology for soil communities is still sparse. A trait-based approach of morphotyping the Collembola community (Chelinho et al. 2013) was applied to enable an extrapolation of the gained results to other soil communities. A natural microarthropod community from a meadow with sandy acidic soil in northern Germany was extracted and introduced to AgNP (10 μg/ kg, 10 mg/ kg) and $AgNO_3$ (10 μg/ kg) contaminated OECD soil. The test was run for 6 weeks under standardized laboratory conditions (12:12 light: dark, 15° C). The experiment was repeated with the same community and changed OECD soil composition to assess how clay and sand content affect the toxicity of AgNP and $AgNO_3$. First results show that the dominant groups of hemiedaphic and euedaphic Collembola and mites benefited from low AgNP concentrations, most probably due to their antimicrobial characteristics which kill off microbes harmful to microarthropods. Higher AgNP concentrations and low $AgNO_3$ concentrations were however harmful to Collembola and mites compared to the control. $AgNO_3$ is therefore more toxic for the dominant groups of microarthropods than AgNP. The number of Collembola individuals and morphospecies was lower after the test than in the initial community. This shows that either the extraction procedure or the change of soil conditions is too stressful for several rare Collembola morphotypes. Mites, dominated by Oribatida, showed a low recovery rate in the control compared to the initial community, indicating that the OECD soil does not offer optimal conditions for mites to thrive. This might be due to the lack of microbial food in the artificial soil. The data analysis of the community test with adjusted OECD soil will be set in comparison to the described results. Currently the same experimental design with OECD soil is being applied to two additional soil communities to assess the impact of AgNP and $AgNO_3$ on additional morphospecies of Collembola and to further validate the methodology.

MO250

A comparison of functional and structural soil testing for risk assessment of plant protection products

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In the development process of a new active substance of a plant protection product (PPP), an insecticide, functional tests and structural tests were performed, which allows a comprehensive assessment of effects on functional and structural soil parameters. In the functional field study – a test on the breakdown of organic matter following OECD GD 56 (2006) and EPFES WS recommendations (2002) – slight transient effects due to the insecticide were found on mass loss of organic matter at 1 month after treatment, while at 3 and 6 months after treatment no significant impact on organic matter decomposition was found. The potential effects of the test substance on structural parameter (soil mites, and collembolans for which a potential risk was identified based on worst-case laboratory testing) were addressed via additional soil samplings and organisms extraction on the same plots of the organic matter breakdown study. Samplings performed at 3 and 5 months after treatment indicated no significant effects on the structural parameters (soil mites and collembolans). In another field study – including higher tested insecticide soil concentrations – with focus on the structure of Collembola populations showed some initial transient effects, but also no longer term impact. On the basis of these results it can be concluded that the functional field test on organic matter breakdown is sensitive and covers the effects on soil mites and Collembola. Sensitivity and initial effects in field tests on organic matter breakdown were also reported in a previous industry-wide ECPA review of such data (Dinter et al 2008). It is concluded that functional test system on organic matter breakdown is sensitive and covering potential effects on soil non-target meso- und macrofauna and considered – due to the direct link to protection goals derived from the EsS concept (plus the robustness of the test conduct itself) a superior testing tool for soil risk assessment of plant protection products.

MO251

Making soil protection goals based on the ecosystem services concept operational in ecotoxicological risk assessments

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What, where, and when to protect are fundamental questions that need to be addressed before designing environmental risk assessment (ERA)schemes. The ecosystem services (EsS) concept can give guidance in what to protect for ERA schemes. Here we use the EsS concept to propose a novel and science based soil risk assessment for PPPs. As a first step we define registration principles followed by identification of soil relevant EsS as a second step. Thereafter, we formulate relevant protection goals (PGs) which are transferred into test systems. Finally our goal will be to define a tiered ERA based on acceptability criteria. The registration principles we identified were that ERA should be workable, scientifically sound and politically acceptable. A well-structured ERA should follow a tiered approach, reduce uncertainties and data gaps concerning organism potentially at risk. A ERA scheme should use validated test systems only, needs to have conservative trigger values, and needs clear acceptability criteria. Soil relevant EsS (e.g. nutrient cycling) can be affected by PPPs. While it is relatively easy to define general PGs, it can be quite challenging to define specific PGs. In the soil area the current focus is on structural PGs (testing of surrogate species) and lacks a clear link to PGs derived from EsS. On the other hand functional PGs directly correlate to EsS. Thus suitable tests based on functional parameters will reduce uncertainties with regard to EsS. Although several functional tests are already available a critical evaluation is needed to select representative and sensitive test systems. Additionally, these novel functional tests need to be linked to information obtained in structural tests for the final risk assessment. The EsS and related PGs might be weighted differently on a spatial scale in agricultural landscapes. For in-crop areas functional aspects are in focus (e.g. food production & nutrient cycling) whereas in off-crop areas structural aspects are additionally of interest. Ecosystem services and the PGs derived from them are suitable to design a new soil ERA scheme. Several EsS are relevant to soil and can be affected by PPPs. Ongoing research will help to define a workable ERA scheme for soil that combines structural and functional endpoints. PGs based on EsS should be addressed by validated and suitable functional tests as these in contrast to structural tests directly link to PGs and thus fulfil registration principles.

MO252

Statistical Power and MDDs in Earthworm field testing

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Earthworm field tests are carried out as part of the risk assessment of plant protection products according to the ISO guideline 11268-3 (ISO 2014). This document provides information on experimental design, test site requirements, data assessment and validity criteria of the test, but does not give indications about the required statistical power of the test or about the effect size that should be detectable in such a field study. In contrast to that, the guidance for summarizing earthworm field studies published by the National Institute for Public Health and the Environment of the Netherlands (De Jong et al., 2006) demands that the minimum effect level that could be detected as statistically significant in a study should be reported. For aquatic test systems, it has been suggested (EFSA, 2013 and Brock et al., 2015) to use the minimum detectable difference (MDD) to evaluate the statistical power of aquatic test systems. In order to provide a better understanding of the earthworm field test we carried out a retrospective power analysis of a series of earthworm field studies covering a range of land uses and soil and climatic conditions using the MDD concept. MDDs will be presented for the overall earthworm population as well as for individual taxa and for ecological groups. The impact of site related (size of the earthworm population, homogeneity of earthworm distribution across the test site) and study design related criteria (e.g. number of replicates) on the statistical power in terms of MDD will be discussed. **Brock TCM, Hammers-Wirtz M, Hommen U, et al., 2015. The minimum detectable difference (MDD) and the interpretation of treatment-related effects of pesticides in experimental ecosystems. Environmental Science and Pollution Research International 22(2): 1160-1174. doi:10.1007/s11356-014-3398-2. De Jong, FMW., Van Beelen, P., Smit, CE. & Montforts, MHMM., 2006: Guidance for summarising earthworm field studies – A guidance document of the Dutch platform for the assessment of higher tier studies. RIVM, The Netherlands, 47 pp. EFSA [European Food Safety Authority] Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters, 2013**. EFSA Panel on Plant Protection Products and their Residues (PPR). Parma, Italy. EFSA J. 11(7): 3290. **ISO, 2014: ISO Guideline 11268-3: Soil quality – effects of pollutants on earthworms. Guidance on the determination of effects in field situations.**

MO253

Soil functional test systems for an in-field soil risk assessment of plant protection products

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Soil functional test systems provide valuable and ecologically relevant information for the risk assessment of plant protection products. Functional tests directly measure ecosystem functions and services which are provided by soils and soil organisms (e.g. organic matter degradation and mineralization). Focusing on structural endpoints in the risk assessment for plant protection products lacks a clear link to the protection goals derived from ecosystem services. Directly measuring soil functions and services can help to better assess the impact of a stressor on the fertility of soils. Furthermore, functional test systems can help to evaluate the ecological relevance of a density change of a soil organism population affected by a certain stressor. In order to improve the current toolbox for the soil risk assessment, a project on soil functional test systems was initiated by the European Crop Protection Association in 2014. In a first step a literature search identified several promising functional test systems which could provide valuable and ecologically relevant information. In a second step a field study was set up in 2015 which measures the impact of two insecticides (Methamidophos, Lindane) on organic matter degradation (minicontainer, bait lamina, litterbag test). Soil micro-arthropod abundances are monitored in parallel to determine the link between effects on the structure of soil micro-arthropods and their soil functional implications (i.e. organic matter breakdown). In this study the suitability of the different functional test systems related to organic matter degradation is evaluated with regard to their potential use in soil risk assessment of plant protection products. The functional test systems (i.e. the minicontainer test) show overall consistent and reproducible results in the field trial. The results indicate that the process of organic matter degradation is dominated by soil microbes. Soil mesofauna contributed only to minor extend to organic matter degradation. Thus, the minicontainer test did not show a clear effect of insecticides on the mesofauna driven organic matter degradation although abundances of Collembola and Acari were heavily reduced by the insecticide applications. Thus, the relevance of structural endpoints on soil micro-arthropods (population/community level) within an in-field soil risk assessment for plant protection products which focus on maintenance of soil fertility (protection of soil functions) is questionable.

MO254

Evaluation of epoxiconazole bioavailability in soil to the earthworm Aporrectodea icterica

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In soil, the determination of total concentration using an exhaustive extraction method is not relevant to evaluate the exposure of an organism to a chemical, because of sorption processes. However, little attention has been paid to the bioavailability of organic contaminants to earthworms, and to their effect on sensitive earthworm species found in cultivated fields, such as *Aporrectodea icterica* [1]. As ecosystem engineers, earthworms influence soil structure and chemistry and are thus key-organisms for the health of terrestrial ecosystems. However, they can be threatened by contaminants such as the fungicide epoxiconazole, which is persistent in soil and presents a chronic toxicity even towards a resistant earthworm species (*Eisenia fetida*) at a concentration close to the recommended dose. This study aimed to propose a mild extraction method to evaluate the bioavailability of the fungicide epoxiconazole to the earthworm *Aporrectodea icterica* [2]. Experiments were conducted in soils presenting various textures and organic carbon contents, spiked with formulated epoxiconazole 7 to 56 days prior to their extraction at one-fold to ten-fold the recommended dose. In parallel, the epoxiconazole concentration was determined in exposed earthworms. All the analysis were performed by UHPLC-MS/MS. The fungicide's effects were evaluated by measuring weight gain, enzymatic activities and total protein contents. The proposed method to evaluate bioavailability was based on the pesticide entrapment in the cavity of hydroxypropyl-β-cyclodextrin, thus fulfilling the criteria of the ISO 17402 norm [3]. Furthermore, this mild method was demonstrated to be sensitive to soil sorption capacities and to ageing. The mild extraction method was then applied to explore the relationship between total and bioavailable concentrations in soil and in *A. icterica*, over 7 or 28 days exposure time. This demonstrated the proportionality between epoxiconazole concentration in earthworm and available in soil (up to 96%, with regression coefficient R^2 0.98). Sublethal effects on earthworm remained not significant. **References** 1. C. Pelosi, S. Joimel, D. Makowski, 2013. Chemosphere 90, 895-900 2. S. Nélieu, G. Delarue, E. Ollivier, P. Awad, F. Fraillon, C. Pelosi, Environ. Sci. Pollut. Res. In Press DOI 10.1007/s11356-015-5270-4 3. ISO 17402, 2008. Soil quality - Guidance for the selection and application of methods for the assessment of bioavailability of contaminants in soil and soil materials

MO255

Ecotoxicological assessment of chemical fumigants utilising an earthworm bioassay (Eisenia andrei) and soil microbial communities

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Fumigation is an important crop protection practice to control agricultural crop pathogens and diseases. Methyl bromide (MeBr) and it success as nematocide since the 1940's is well documented. It has, however, been identified as ozone depleting gas and it was to be phased out by 2005 under the Montreal Protocol (2002). The more common alternatives to MeBr include metham sodium (MNa) and cadusafos but very little information is available about their effects on non-target and beneficial soil organisms. The aim of the study was to determine the ecotoxicity of MNa and cadusafos utilising a standardised earthworm (*Eisenia andrei*) ecotoxicity test (OECD 2004). In addition to this the genotoxicity of MNa and cadusafos was assessed by investigating DNA damage (comet assay) of individual earthworm cells. Further, to assess changes in the microbial community function and structure by means of Biolog™ Ecoplates and phospholipid fatty acid (PLFA) analysis after treatment of soil with chemical fumigants. The chemical fumigants had a marked negative impact on the survival, growth, reproduction and the genotoxicity of the earthworms with MNa causing greater harm than cadusafos. The effects on the microbial community varied. Both chemicals had an inhibitory effect on the microbial growth in terms of the viable biomass determined by PLFA and the average well colour development in the Biolog™ Ecoplates. No lasting effects were observed in the community structure. Overall, cadusafos had a more pronounced effect on the microbial community functional diversity than MNa.

MO256

Application of Equilibrium partitioning-based model framework for evaluating soil and sediment hazards of lipophilic nonpolar organic substances

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The target lipid model (TLM) is a QSAR framework used to predict acute and chronic toxicity of substances based on structure. This framework has been extended for calculation of predicted no effect concentrations (PNEC) of highly lipophilic hydrocarbon substances to support risk assessment activities of single chemicals as well as complex substances such as fuels, solvents, and lubricants. Recently, this framework was extended into soils and sediments using equilibrium partitioning (TLM-EqP) to support hazard evaluation, risk assessment, and read across activities thereby maximizing use of available data. The TLM-EqP

framework established the range of sensitivities for common test species (invertebrates, microbial endpoints, plants), which supports read across from the results of one assay to another. This framework was applied to recent dossier updates to address existing data gaps. The approach consisted of a weight of evidence built using TLM-EqP predictions and read across to available data, which allowed an improved experimental design for additional testing. This framework was used to establish the upper limit of the predicted porewater solubility (~100 mg/kg in bulk soil) to avoid potential formation of oily residues, which introduce the potential for physical oiling. While the mechanism of physical oiling could be a true hazard, predicted environmental concentrations in soil for most substances under typical use patterns are very low (< 0.01 mg/kg). Confirmatory testing in soil and sediment showed lack of toxicity consistent with model predictions and consistent with existing test data in water and soil. This framework promotes realism in chemical risk assessments by designing tests based on physicochemical properties and likely hazards of the test substance. This presentation will review the technical basis for the framework and illustrate the application of the methodology to available case studies, i.e. dossier updates to insoluble, nonpolar organic substances.

MO257

Effects of wheat seed dressings with neonicotinoid insecticides and fungicides on soil organisms

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Seed dressing with pesticides is widely used to protect crop seeds from pest insects and fungal diseases. While there is mounting evidence that especially neonicotinoid seed dressings detrimentally affect pollinators, surprisingly little is known on potential side effects on soil biota. We hypothesized that soil organisms would be particularly susceptible to pesticide seed dressings as they get in direct contact with these chemicals. Using mesocosms with field soil we investigated, whether seeds treated either with neonicotinoid insecticides or fungicides influence the activity of earthworms, collembolan, protozoa and microorganisms. Additionally, we also examined interactions between soil organisms and seed dressings. The full-factorial design consisted of the factor seed dressing (control vs. insecticide vs. fungicide), earthworm (no earthworms vs. addition of *Lumbricus terrestris* L.) and Collembola (no Collembola vs. addition of *Sinella curviseta* Brook). We used wheat seed material (*Triticum aesticum* L. cf. Lukullus) available for farmers at a recommended seeding density of 367 m^{-2} (18 seeds per mesocosm). Seed dressings (particularly fungicides) increased Collembola surface activity, increased the number of protozoa and reduced soil decomposition rate but did not appear to affect earthworm activity. Earthworms decreased wheat growth, reduced soil basal respiration and microbial biomass but increased soil water content and electrical conductivity. Collembola increased earthworm surface activity but reduced soil basal respiration. Earthworms interactively affected effects of seed dressings on Collembola activity. We find it remarkable that the reported non-target effects of seed dressings on soil organisms were found after a one-time application of only 18 pesticide treated seeds. Because of the fundamental role of soil organisms in agroecosystems, investigations on their interactions with pesticides should receive more attention.

MO258

Sublethal effects of epoxiconazole on the earthworm Aporrectodea icterica

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Earthworms play a key role in agroecosystem soil processes. This study aims to assess the effects of different doses of a commercial formulation of epoxiconazole (Opus®), a persistent and widely used fungicide, on the earthworm *Aporrectodea icterica*. A laboratory study was conducted in a natural soil in order to measure effects of Opus® on earthworm mortality, uptake, weight gain, enzymatic activities (catalase and glutathione-S-transferase), and energy resources (lipids and glycogens). The estimated LC50 was 45.5 mg kg^{-1}, or 268 times the recommended dose. Weight gains were 28, 19, and 13 % of the initial weight after 28 days of exposure in the control and D1 and D10 (1 and 10 times the recommended dose) treatments, respectively. No difference was observed for catalase activity between the three treatments, at 7, 14, or 28 days. The glutathion-S-transferase (GST) activity was two times as high in D1 as inD0 at 14 days. At 28 days, glycogen concentration was lower in D10 than in the D1 treatment. This study highlighted moderate sublethal effects of the commercial formulation Opus® for earthworms. Considering that these effects were observed on a species found in cultivated fields, even at recommended rates, much more attention should be paid to this pesticide. **Keywords:** Ecotoxicology, Fungicide, Biochemical biomarkers, Mortality

MO259

Poplar-assisted bioremediation of a PCB historically contaminated soil in Southern Italy

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The plantation of selected plants in contaminated sites can support organic contaminant degradation. Plant roots can release exudates and supply nutrients useful for autochthonous microorganisms, which in turn enhance their biological activities and can degrade xenobiotics. A synergistic action between rhizosphere microorganisms and plants can lead to an increase in bioavailability of hydrophobic compounds, making them more susceptible to degradation. The results of the first 30 months of a poplar-assisted bioremediation application to a multi-contaminated (PCBs and heavy metals) soil in Southern Italy are here reported. The investigated site has been used for several decades as an unsupervised waste disposal. A specific poplar clone (Monviso), was applied in an area of 785 m^2 of the contaminated site where six hundred poplar cuttings were planted. Sampling were performed 14 and 30 months after the poplar-plantation. At each sampling, soils at different depths and distance from the tree trunk, tree roots and leaves were collected for chemical and microbiological analysis. PCB and heavy metal (HMs) analyses were performed using a GC-MS and an ICP-MS, respectively. Microbial analysis were carried out to assess total microbial abundance, cell viability and dehydrogenase activity (DHA) of the autochthonous microbial community. The comparison of the chemical and microbiological analyses performed at day 0 and 14 and 30 months after the poplar plantation, show a decrease in soil of PCBs under the Italian legislation limits and also a significant decrease in soil of all HMs. The poplar plantation showed its effectiveness both in promoting the persistent organic contaminant degradation and in the phyto-containment of the inorganic ones. In line with these results microbiological activity increased significantly in the rhizosphere soil.

MO260

Re-calibration of the earthworm tier 1 risk assessment of plant protection products

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To address potential risks of plant protection products to earthworms, a risk assessment is required. This risk assessment comprises two steps 1) deriving no-observed effect levels (NOEL) from laboratory reproduction tests and applying a trigger value, to cover uncertainties, and if this indicates a potential risk, 2) conducting field studies. In this review paper the tier 1 earthworm risk assessment for plant protection products is calibrated by comparing the NOEL in laboratory earthworm reproduction tests with effect levels on earthworm populations under realistic field conditions. A dataset of 54 pairs of studies conducted in the laboratory and in the field with the same plant protection product was compiled, allowing a direct comparison of relevant endpoints. The results indicate that a tier 1 assessment factor (AF) of 5 combined with a regulatory relevant soil layer of 0-5 cm provides a conservative tier 1 risk assessment. A risk was identified by the tier 1 risk assessment in the majority of the cases at application rates which were of low risk for natural earthworm populations under field conditions. Increasing the conservatism in the tier 1 risk assessment by reducing the depth of the regulatory relevant soil layer or by increasing the tier 1 AF would increase the number of false positives and trigger a large number of additional field studies. This would however not increase the margin of safety for earthworm populations. The analysis revealed that the risk assessment is conservative if an AF of 5 and a regulatory relevant soil layer of 0-5 cm are used.

MO261

Determination of Soil Adsorption Coefficient (Koc) Values for a Series of Aliphatic Alcohols using the OECD 121 HPLC Method

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A study has been performed to generate experimental data for the organic carbon-water adsorption coefficient (Koc) of a range of alcohols (C6-C16) using an accepted OECD method (OECD 121). This Test Guideline describes an experimental method using High Performance Liquid Chromatography for the estimation of the adsorption coefficient Koc. The HPLC method is an estimation method based on correlation of retention data with true Koc values determined in soil/sediment/sludge-water systems. A series of reference substances, as recommended in the OECD 121 guideline, were assessed using a HPLC system under the specific conditions outlined in the method. The retention data were used to generate a correlation plot of literature adsorption coefficient (log Koc) values and the log capacity factor (k) for the reference substances. A range of linear alcohols, ranging from C6 (1-hexanol) to C16 (1-hexadecanol), was subsequently run under the same HPLC conditions and their capacity factors (k) generated. The calibration line (log k vs log Koc) for the reference substances was then used to generate the estimated log Koc values for all the individual linear alcohols. Applying the OECD 121 guideline method, the HPLC-derived log Koc values for these alcohols ranged from 1.25 (1-hexanol) to 5.40 (1-hexadecanol). When taking into account other experimental log Koc data from sewage sludge adsorption studies (n = 4) and a recent OECD Guideline 106 test for 1-decanol, very good agreement with HPLC screening data was obtained. The HPLC log Koc value for 1-decanol was within a factor of 2 compared to the value obtained from the OECD 106 study which demonstrates the accuracy of the technique. However, QSAR predictions of log Koc vary widely in comparison to HPLC and measured data depending on which QSAR method (regression-based log Kow or fragment-based Molecular Connectivity Index) is selected. This study demonstrates the good performance of the HPLC screening method for determination of log Koc of aliphatic alcohols without the complications associated with definitive experimental determination using the OECD 106 method due to their rapid biodegradability. An evaluation of the various QSAR based methods for prediction of log Koc for aliphatic alcohols is also discussed.

MO262

How representative is Eisenia andrei as a standard species for soil ecotoxicological testing?

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The widely distributed composting earthworm species *Eisenia andrei* is recommended and used for ecotoxicological testing. However, the scientific literature abounds with scepticism about its suitability and its representativeness because it is a litter-dwelling species representing a totally different ecotype than most other soil dwelling oligochaetes. To address the question whether toxicity data obtained with *E. andrei* could be extrapolated on reasonable grounds to other earthworm species, we compared the sensitivity of *E. andrei* and four other species representing different ecotypes. *E. andrei, Aporrectodea trapezoides, Amynthas diffringens, Perionyx excavatus* and an indigenous *Chilota* species were exposed under similar conditions to a concentration series of copperoxychloride as prescribed by OECD guidelines for toxicity testing. We monitored mass change and cocoon production over time and compared body copper accumulation at the end of the exposure. No worms in the uncontaminated control soil or in the 20mg/kg exposure lost significant body mass. With exception of *A. trapezoides* no other species lost significant mass at 80 and 160 mg/kg exposures. At 320 mg/kg exposure significant differences occurred between species with *E. andrei* and *P. excavatus* (the two litter-dwelling species) seemingly more robust. Cocoon production differed between the composting and soil dwelling species with the latter two species being more severely affected. Body concentrations of Cu increased similarly with increasing exposure concentrations in all species. At the lower, environmentally more relevant concentrations mass change and body load of Cu were not strong discriminating factors. However at the higher concentrations it clearly affected the soil dwelling species more detrimentally. Cocoon production was a more sensitive endpoint than body mass change and a distinction between the soil dwelling and the litter dwelling species was obvious at lower concentrations. The simultaneous onset of the detrimental effect of copperoxychloride on cocoon production (albeit at different levels of severity) in all species provided evidence that extrapolation to other species may be warranted, provided that the results are interpreted with the necessary caveat that the intensity of effects may differ as a result of differences in habitat type and lifestyle.

MO263

Monitoring the residues of currently used pesticides in agricultural soils - the first step of the post-registration control

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Data on currently used pesticides (CUPs) in surface and ground water are relatively abundant due to ongoing water monitoring in many EU countries. Several CUPs or/and their degradation products have been repeatedly found at high frequency and also at considerable concentrations. Water monitoring scientists have hypothesized that the secondary source of CUPs in water are soils in watershed that keep the residues for long time and slowly release them (or their degradation products) to ground/surface water. However, there are surprisingly no data available for CUPs in soils in EU to work on this hypothesis. Not only for the purpose above, but also for the post-registration control of approved plant protection products, the monitoring of CUPs in Czech agricultural soils was performed last year. Fluvisols (alluvial soils) were selected for the study as they are intensively used for agriculture and actively communicating with surface/ground water. Soil samples were collected in February to March to address the "long-term" ("oldest possible") residues before new pre-emerging applications and to avoid the peaks caused by recent applications. In 75 soils, 65 active ingredients were determined by multiple residue method using QuEChERS extraction and UPLC-MS/MS detection. The results identified several CUPs frequently found above LOQ. The most frequently detected compounds were azole fungicides epoxiconazole (36% of samples), tebuconazole (27%), flusilazole (17%), prochloraz (16%), and propiconazole (10%). Their mean concentrations in positive samples were about 0.01 mg/kg. The second most frequently detected compound was 2-hydroxy atrazine (29%) with mean concentration in positive samples about 0.03 mg/kg and the highest measured concentration of 0.135 mg/kg. This finding is surprising as atrazine was banned in CR in 2006. It can also explain still frequent detections of atrazine degradation products in water monitoring. Other CUPs with frequent findings were fenpropidin (15%), diflufenican (13%), terbuthylazine (13%) and others. The results also show how complex is the mixture of CUPs in current agricultural soils - half of the soils had 5 or more CUPs detected. The soil residues of CUPs should be definitely considered and soil monitoring programs for CUPs should be initiated in EU countries along with water monitoring. Financial support was obtained from GACR project 15-20065S.

MO264

PERSAM PECsoil calculation and pesticides risk assessment: support or burden for regulators?

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Pesticide risk assessment for soil organisms is currently performed by calculating the toxicity/exposure ratio, that is the comparison of the toxicity that a specific pesticide has for a soil not-target organism and the pesticide exposure predicted for that same organism. The substance toxicity is assessed with targeted ecotoxicological studies on certain representative species, while exposure is assessed by calculating a Predicted Environmental Concentration (PEC) with models. According to the new EFSA Guidance Document for predicting environmental concentrations of active substances of plant protection products and transformation products of these active substances in soil (EFSA Journal 2015;13(4):4093) the new model PERSAM has to be used for the calculation of pesticides PEC_{soil}. This model is structured in five Tiers with different levels of complexity. Tier 1 is a very conservative one while the higher Tiers, even if closer to realistic conditions, are quite complex and time-consuming since the entire calculation process is quite slow and long-lasting. This work addresses such a complex exposure assessment in the context of the standard evaluation procedure that regulators have to deal with in their activity of registration of both active substances and PPPs. In particular, attention will be focused on how often a higher tier results necessary and how often Tier 1 may be used to exclude pesticide of low risk for soil non-target organisms. A subset of Plant Protection Products (insecticides and fungicides, the substances that generally may pose some risk to soil non-target organisms) authorized at National level has been considered. Ecotoxicological endpoints on soil arthropods and earthworms (NOEC and EC_{50}) have been taken mostly from EFSA Conclusions and Review Reports and recorded in an MS Excel spreadsheet. RAC (Regulatory Acceptable Concentrations) have been derived from these endpoints. PEC_{soil} for the selected active substances have been calculated with PERSAM and with the simple tier 1 presently used in registration and then compared with the RAC. The percentage of active substances which showed an unacceptable risk for each Tier has been calculated, and comparison between the old system and the new one has been presented together with some considerations on the efficacy, reliability and usefulness of all the PERSAM tiers.

MO265

From bioavailability science to regulation of organic chemicals: a communication experiment

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The bioavailability of organic chemicals (i.e., mainly industrial chemicals, pesticides, biocides and pharmaceuticals) in soil and sediment is an important area of scientific research. However, this area remains only partially recognized by regulators and industries working in the environmental sector. Based on the positive experiences already made with metals, regulators have recently started to consider bioavailability within retrospective risk assessment (RA) frameworks (e.g. of contaminated sites) for organic chemicals. By doing so, realistic decision-making in terms of hazard definition and priority considerations finally resulting in optimised cost allocation can be achieved, rather than relying on the established approach of using total-extractable concentrations. However, implementation of bioavailability remains difficult because scientific developments on bioavailability are not always translated into ready-to-use approaches for regulators and, therefore, no integrated approach for implementation is available. For the same reason, bioavailability remains largely unexplored within prospective regulatory frameworks (e.g. REACH) that address the approval and regulation of organic chemicals. To facilitate the inclusion of bioavailability within more realistic RA frameworks, agreement and common understanding between scientists and regulators is required. This contribution has been prepared by the five proposing authors that represent a bigger group of authors from academia, industry and regulation, who have recently arrived at an agreement and discussed bioavailability concepts and methods. In addition, we offer a simple, pragmatic and justifiable approach for use within retrospective and prospective risk assessment. A key motivation for this communication exercise was the differences between scientific and regulatory perception. The developments achieved at the Special Science Symposium focused on bioavailability, organized in 2014 by SETAC Europe (http://sesss10.setac.eu/), subsequently led to a featured paper (Environ. Sci. Technol. 49:10255-10264, 2015). In the paper, we propose a simplified approach in which the assessments of soil/sediment and the target chemicals should be based on two measurable values: the total extractable concentration, and the bioavailable concentration as measured with robust and reproducible chemical and/or biological methods (desorption extraction, passive sampling, aqueous extraction, and/or biological tests).

MO266

General overview of experimental terrestrial toxicity studies submitted in the framework of the REACH Regulation

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The REACH Regulation entered into force in June 2007. Since then European Chemicals Agency (ECHA) has received a large amount of scientific and administrative information related to chemical substances submitted in the registration dossiers. To avoid unnecessary testing, the REACH Regulation provides registrants with the possibility to build testing strategies and to adapt the standard information requirements based on the specific conditions listed in the Regulation. In order to understand what type of data on terrestrial ecotoxicity were submitted in REACH registration dossiers, a detailed analysis of the availability and content of relevant information was performed. The similar analysis was performed after the first registration deadline (Versonnen et al., 2013). The current analysis covers the data submitted for the two registration deadlines (1 December 2010 and 1 June 2013) and new experimental data produced as a consequence of the evaluation of testing proposals and the compliance checks. As in the previous analysis, both the most used test guidelines and test species were investigated. As the outcome of the conducted analysis, it can be concluded that for soil invertebrate testing mostly standard guidelines were used and consequently, a clear prevalence has been detected for testing on the species recommended by the standard test guidelines. Nevertheless, the reporting included a large variety of species from very different families. For terrestrial plants, the most extensively reported test guidelines were OECD 208, ISO 11269-1 and ISO 11269-1. It is important to stress that for many cases, the information on the test guideline according to which the study was conducted, was not reported or was not reported correctly. Moreover various adaptations and waiving justifications were used to omit the experimental testing for terrestrial toxicity. When interpreting the findings of the current analysis, it should be noted that in principle the current results may be affected by the outcome of the dossier evaluation works performed by ECHA. By November 2015, ECHA had issued more than 100 final decisions, which requested terrestrial toxicity studies to be performed according to standard test guidelines. The analysis of the terrestrial ecotoxicity data submitted by registrants to fulfil REACH data requirements will be presented.

MO267

Natural transformation of chlordecone into 5b-hydrochlordecone in French West Indies soils: statistical evidence for investigating long-term persistence of organic pollutants

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Chlordecone (CLD) was an organochlorine insecticide whose previous use resulted in an extensive pollution of the environment with severe health effects and social consequences. A closely related compound, 5b-hydrochlordecone (5b-hydroCLD), has been searched for and often detected in environmental matrices from the geographical area where CLD was applied. The current consensus considered that its presence was not the result of a biotic or abiotic dechlorination of CLD in these matrices but rather the consequence of its presence as impurity (synthesis by-product) in the CLD released into the environment. The aim of the present study was to determine if and to what extent degradation of CLD into 5b-hydroCLD occurred in the field. To test this hypothesis, the ratios of 5b-hydroCLD and CLD concentrations in a dataset of 810 soils collected between 2006 and 2012 in Martinique were compared to the ratios measured in representative samples of commercial formulations. The statistical analysis highlights the unexplained high amount of 5b-hydroCLD in soil associated with the CLD spread. The hypothesis proposed for explaining why 5b-hydroCLD/CLD ratios in soils exceed the corresponding ratio in commercial formulation leads to the inverse demonstration: 5b-hydroCLD should be preferentially eluted from soil by water and may be metabolized, strengthening the significance of the statistical evidence demonstrating that 5bhydroCLD amounts in field soil cannot be justified by its sole input as an impurity present in the Kepone® and Curlone® commercial formulations. The bishomocubane structure of the compounds invalidates all the other origins unless CLD. Results showed a significant increase of the 5b-hydroCLD/CLD ratio in the soils—25 times greater in soil than in commercial formulations— when the investigations dealing with the differential transfer of 5b-hydroCLD compared to CLD lead to promote a decreasing mass ratio in situ compared to the formulation, the opposite of the trend observed. This suggested that natural CLD transformation into 5b-hydroCLD over the long term occurred in these soils. Previously, considered as a by-product due to CLD synthesis, 5b-hydroCLD turns to be a highly probable dechlorination product of CLD. However, the details on 5b-hydroCLD formation are still to be determined although some potential pathways for this formation could be advanced. Results from this study may impact future decisions for the remediation of the polluted areas.

MO268

Toxicity depends on the life stage: proposal for a new testing method using Folsomia candida

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Toxicity of pollutants depends on the life stage of the test organisms. Different sensitivities between juveniles and adults on earthworms have been reported, meaning that often juveniles are more sensitive than older stages. Current test methods seldomly cover this aspect and may underestimate toxicity. Hence the development of new test methods to assess the environmental risk of contaminants is of high interest. The aim of this study was to develop and assess a novel test method using the standard test species *Folsomia candida*. The current OECD test is standardised for the exposure of juvenile organisms being 10-12 days old. In the present study the toxicity of Cd was compared between 3 different developmental stages: eggs, juveniles (10-12 days) and adults (21 and 28 days old). Results showed that Cd was not affecting significantly the hatching success of *F. candida* at 60mg/kg (the reproduction EC50), but survival of juveniles was affected as well as reproduction. These effects were stronger compared to the results of 10-12 days old organisms. Exposure of adults to Cd showed less effect in terms of reproduction compared to those effects observed after exposure of juveniles. The present assessment of effects at different life stages allows to identify the stageat which the effects occurred. Moreover, exposure of eggs enhanced the effects in terms of reproduction compared to exposure of juveniles and adults.

MO269

Effects assessment on terrestrial organisms: Integrated testing strategy under the REACH Regulation

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The scope of the terrestrial effect assessment under REACH is restricted to soil non-vertebrate organisms living the majority of their lifetime in the soil and being exposed to substances through the soil pathway. Under REACH framework risks to predators from the secondary poisoning due to chronic exposure to a substance through the terrestrial food chain should also be assessed by registrants. The REACH “standard information requirements” are listed in Annexes VII to X to the REACH Regulation. The minimum dataset depends on the annual tonnage (tpa), i.e. quantity manufactured or imported by a registrant per year. This principle also applies to the standard terrestrial toxicity data necessary to be provided in the REACH registration dossier. For registrations from 100-1000 tpa, results of short-term toxicity studies on soil invertebrates and plants should be provided together with the results of toxicity study for soil microorganisms. When the registration tonnage is above 1000 tpa, long-term toxicity studies with soil invertebrates and plants should be provided. For highly adsorptive (log Kow >5) and very persistent substances (half-life in soil >180 days), long-term toxicity data instead of short-term data should be considered even at registration tonnage from 100-1000 tpa. Column 2 of REACH Annexes IX and X lists specific rules for adaptation of the “standard information requirements”. In case of terrestrial toxicity data, the studies do not need to be conducted if direct and indirect exposure of the soil compartment is unlikely. Besides, the equilibrium partitioning method (EPM) may be applied to assess the hazard to soil organisms. The principles and practicalities of soil hazard assessment are summarised in ECHA's *Chapter R7c Guidance*. It provides the integrated testing strategy for assessment of effects on terrestrial organisms. ECHA in co-operation with other REACH stakeholders has processed a number of dossier evaluations where various issues of terrestrial effect assessment were addressed. Based on these discussions, the screening assessment approach for soil, the criteria to define substances with high potential for adsorption and substances very toxic to aquatic organisms were further clarified. Moreover, the threshold given in some standard soil toxicity test guidelines was questioned and respective analysis to identify new relevant parameter(s) and threshold(s) to define volatile substances for the purpose of soil toxicity testing was performed.

Risk Assessment of Biocides - latest developments (P)

MO270

Example of a conceptual garden scenario and potential for ecosystem services and ecological modelling to support the regulatory process

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At the present time, outdoor risk assessments of biocidal consumer products are typically based on the impact they have on the immediate treated area. Recovery at the scale of the garden, rather than the treated soil, is not generally considered when reviewing the risk and benefit of the product. This poster aims to demonstrate how treated area and the garden itself could be considered as a part of a larger urban environment, where the fraction of impacted soil can be balanced with the potential for recovery. In addition, consideration should be given to the role of the product and the ecological relevance of the treated area with respect to the urban environment. Finally, the importance of a consensus on the ecological relevance of urban area is highlighted, in order to provide effective guidance to risk assessors.

MO271

Aspects of scoping and running environmental risk assessment for a Biocidal Product Family

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The Biocidal regulation requires an environmental risk assessment (ERA) to be performed on the active substance (AS), for the precursor(s) of in-situ generated AS and for any substance of concern (SoC) present in any biocidal product (BP) to be marketed on the EU market. In the meantime, the regulation allows the creation a biocidal product family (BPF) in order the to facilitate a group of products to access the market. Within a BPF, all BP have the same AS and have similar uses. Variations in the composition, replacement of non-active substances is allowed, providing that change do not affect the level of risk or reduce the efficacy. The number of BP and AS in a BPF is not defined. A simple and straightforward BPF includes a single AS and omits any SoC. However, more complex BPF may be built and include more than one AS and SoC for environment. Since the assessment of the BPF shall consider the maximum risks to the environment over the whole potential range of products within the BPF, preliminary risk assessments is needed to define the scope of ERA (i.e. compartment at risks, composition leading to maximum risks). The initial step is to gather all necessary information for running preliminary risk assessment: -Product Type, detailed information on use claimed -Identification of relevant emission scenario document. However, for many biocide uses, no ESD have been developed and will require tailor-made scenarios to be performed -Quantitative composition and variation of components within the BPF -Collecting hazard data for all components including PNEC values Which methodology to be applied for running the risk assessment? Various methods have been proposed to assess the risk of mixture for the environment. This includes e.g. component-based approach. Among the modelling tools, EUSES has been extensively used for the assessment of risks posed by AS under the review program of existing AS. The methodology is quite clear for assessment of BPF containing a single AS, the picture much more complicated with combination of AS and SoC. A transitional guidance has been made available by ECHA. This poster will address a hypothetical case study with BPF containing more than one AS and relevant substance to assess together with a combination of wide dispersive use. The purpose of this work is to initially review the issues with ERA for BPF, the need for an adaptable methodology able to cover complexities associated with variation of composition and uses.

MO272

Replacement possible for biocides with carcinogenic formaldehyde

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The National Institute of Public Health and Environment (RIVM) made an inventory of the use of formaldehyde in disinfectants and preservatives (biocides) and the availability of alternatives. There seem to be sufficient alternatives available for the majority of disinfectants and preservatives containing the carcinogenic substance formaldehyde. However, the suitability of these alternatives needs to be reviewed per sector and application. Formaldehyde is the active substance in many disinfectants and preservatives. It is expected to be classified as carcinogenic by 1 January 2016 at European level. This can have implications for the use of biocides containing formaldehyde that are currently on the market. In the Netherlands, the disinfectants and preservatives that are made available on the market are assessed by the Board for the Authorisation of Plant Protection Products and Biocides (Ctgb) and authorised as safe when used according to the product label. These products are used by professionals only. There are alternatives for the following applications: cleaning and disinfection of surfaces and instruments in the healthcare sector; disinfection of indoor public areas; disinfection of stables and animal housing; preservation of detergents and cleaning products; preservation of paints, varnishes, inks and other industrial liquids, preservation of liquids in cooling systems and for slime control in the paper and pulp industry. For some applications, very few alternatives were found. This lack concerns, for example, the disinfection of mushroom-growing rooms, litter bins for sanitary towels, footwear and cattle hooves. This also applies to some preservation products, such as in lubricants, metalworking fluids, and the preservation of human and animal corpses and biological tissues. The Dutch Ministry of Infrastructure and the Environment will use the inventory to review – in collaboration with industry branches – whether there will be bottlenecks when the use of formaldehyde is further restricted. Also, the study will be used to inform users about the need to restrict exposure to formaldehyde and formaldehyde emissions as much as possible. This is in line with the current policy of the Ministry of Infrastructure and the Environment for priority substances of concern (ZZS substances). The Ministry aims to substitute the use of formaldehyde with less hazardous substances and to stimulate the use of non-chemical alternatives.

MO273

Harmonizing the environmental risk assessment for antifouling paints

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Active substances (biocides) to be used in antifouling paints are authorized on a European level using a harmonized risk assessment method. An active substance can be authorized in Europe if the use on commercial sea ships does not lead to unacceptable effects on the ecosystem immediately outside a commercial seaport. After an active substance is authorized, industry has to apply for national authorization of the formulated antifouling paints to bring such products on the market. There is an obligation of mutually acceptance of authorizations between European member states, but the product authorization methods are not yet harmonized. Some countries have no authorization method at all, and where methods exist, they differ in several aspects. The absence of a harmonized approach hampers the free movement of goods and may eventually lead to a shortage of suitable products. In this respect, it should be noted that antifouling paints also provide social and environmental benefits, by reducing fuel consumption and preventing the spreading of invasive species. Besides ship yards will suffer economic damage when companies move outside the European Union because effective antifouling paints can no longer be applied here. To solve this problem, the Netherlands initiated the process in which European member states and stakeholders will work to develop a harmonized approach for national authorizations. Agreement is needed on which type of ships and harbors should be included (commercial or pleasure crafts), which part of the aquatic environment is to be protected (only marine waters or also freshwaters, outside or also inside harbors), and how to include the risks of application, maintenance and repair in the risk assessment. A further step is to develop exposure models that are harmonized with respect to e.g. dimensions and hydrology of the harbor and surroundings, the number and size of ships at berth and moving, and the fraction of ships treated with antifouling paint. In addition, the methodology should account for the fact that environmental characteristics (salinity, temperature, pH, DOC, etc.) differ between countries. Other issues are how to account for market share, and how to deal with mixtures of active substances. In the end, the harmonized approach should deliver estimates of exposure that represent a 'realistic worst case' for different countries or regions. The first results will be presented at the conference.

MO274

Testing antifouling sealers efficiency and toxicity

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Application of antifouling paints (AP) is the method most commonly used to prevent the development of marine biofouling on boat surfaces. Leakage of toxic agents from AP on boat hulls is one of the main sources of biocide release into water bodies. The scale of the problem is easily appreciated taking into account that 6 million boats move within Europe and components of AP, such as copper, zinc, irgarol and TBT, can be highly toxic to the environment. Application of multiple layers of AP onto boat hulls over the years is a common practice among boat owners, which has lead to the maintenance of banned paint components, such as TBT, on boat hulls. The application of new layers of AP on boat hulls is normally preceded by the use of sealer coats (SC), which are developed to prevent product incompatibilities and leaching from older paint layers. Therefore, the efficiency of such SC is of importance if leaching from older paint products to the environment is to be avoided, especially nowadays with the increase usage of mechanical brushing devices for removal of biofouling from boat hulls. In this study, manipulative laboratory based experiments tested the performance of five SC from leading paint producers. Sealer coats efficiency in locking underlying AP, its resistance to brushing action and toxicity to aquatic organisms were evaluated. The toxicity of SC leachates produced in 7‰ natural seawater was tested on four organisms, the bacterium *Vibrio ficheri*, the alga *Ceramium tenuicorne*, the crustacean *Nitocra spinipes* and the gastropod *Theodoxus fluviatilis*. All SC showed to be toxic, causing increased mortality, inhibition of growth or bioluminescence inhibition, to at least one of the tested species. In terms of locking efficiency, none of the SC showed to be able to successfully lock biocide release from all the AP tested. Locking capacity of SC was dependent on the underlying layer of AP being tested and identity of the metal being released, with brushing action generally reducing locking efficiency of SC. Our results emphasise the need to improve the locking efficiency of SC in order to prevent release from underlying AP. Moreover, we showed that the use of mechanical brushing devices on SC is also not recommended as it reduces SC locking capacity and consequently increases leaching rates from underlying paints. As SC leachates were toxic to tested organisms, the use of biological tests in the regulatory process of such products is recommended.

MO275

Emissions and photo-transformation of biocides on building facades using the example of terbutryn

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Biocides as triazines or isothiazolinones are added to render and paint in order to protect building facades from microbial deterioration. Previous studies have shown that biocides leach out of the material during rain events and can be detected in the urban environment. In the present study the leaching of terbutryn from artificial walls equipped with two types of render (acrylate and silicone resin render) was observed for 19 months. On the one hand, the emissions of terbutryn (concentration and mass load) were determined; on the other hand, photodegradation products were identified and studied in the leachate as well as the render. It could be shown that biocides leach predominantly within the first months of the facade lifetime. While the leaching was determined by driving rain within this period the leaching was reduced afterwards and determined by other factors. Several photo-transformation products could be detected in the façade runoff. However the major fraction of the transformation products was still remaining in the façade material. Based on the amount of terbutryn and its transformation products in the leachate as well as remaining in the material, the overall mass balance can be closed. This study showed, that the focus on transformation products during the assessment of biocides in building material is of high importance: not only concering the emissions during life-time, but also when it comes to disposure of the coating material as waste.

MO276

Non-monotonic dose-response relationships for toxicity of the biopesticide DiPel ES on Daphnia magna

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Currently, there is a trend to increase the use of supposed environmentally friendly biopesticides. In this context, *Bacillus thuringiensis* (BT) pesticides, such as DiPel ES, are amongst the worldwide most used to control caterpillars. BT produces endotoxins that cause effects described as highly specific to some insects. These biopesticides are used worldwide without environmental limits because they are considered to present negligible toxicity to non-target organisms. This study investigated the toxic responses of the non-target organism *Daphnia magna* to waterborne DiPel ES. Neonates were 48h-exposed to DiPel ES for LC50 and EC50 estimation. *D. magna* 17-21 days old were similarly exposed for EC50 and biomarkers. The biomarkers evaluated (whole body) were body weight, global protein, chitobiase, catalase, glutathione reductase, glutathione S-transferase, and acetylcholinesterase. Particle size distribution in the exposure media was determined by preliminary measurements.Dipel affected immobilisation and mortality of neonates ($p>0.001$, EC50=from 0.17 to 0.20μL/L, LC50=0.40 to 1.17μL/L). Similar sensibility was found for immobilisation of adult daphnids (EC50= from 0.17 to 0.29μL/L), without significant mortality. Mortality and immobilization endpoints displayed a biphasic and inverse U-shaped response. All biomarkers but acetylcholinesterase were affected by DiPel ES exposure ($p< 0.05$), displaying multiphasic dose-responses. This suggests a mechanism of toxicity able to affect generally organs and systems of the whole organism. The main particle size in experimental media was compatible to bacteria spore, however a secondary particle size compatible to metabolic active BT, was observed when toxicity was occurring. Therefore, it is possible that chemical and physiological interactions resultant of different BT activity at various DiPel ES concentrations might be related to the observed non-monotonic effects. The core assumption of current toxicology is that toxicity increases monotonically with contaminant exposure. However, the present results challenge the universal applicability of this central ecotoxicological principle. Further studies are required to isolate the cause of non-monotonicity of this BT biopesticide.

MO277

Secondary poisoning risk assessment for insectivorous species under Biocidal Products Regulation: case of insecticides used against ants

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The risk of secondary poisoning is a part of the environmental risk that has to be assessed under the Biocidal Products Regulation 528/2012. It relates to toxic effects occurring in higher levels of food chains which result from ingestion of contaminated organisms from lower trophic levels. Insectivorous animals have potentially a risk of secondary poisoning through eating treated insects, especially during the outdoor use of insecticidal products, like those against ants. A harmonized approach to assess the secondary poisoning risk for insectivorous birds or mammals is actually available in the Exposure Scenario Document for household and professional insecticides (ENV/JM/MONO(2008)14). However, this approach based on the SANCO/4145 (2002) EU guidance for Plant Protection Products is relevant only for spray products and not for other type of application such as gel bait often used against ants. This poster describes an example of biocidal use for which an exposure scenario is currently not available. It presents first reflections on the parameters which could be considered for the construction of a new model of exposure more specific to gel bait product against ants. Considerations related to the type of product, its mode of action, specificities of target (here ants) and predator species (behavior, biology) are exposed and discussed for a future scenario that could better reflect the realistic worst case of secondary contamination of insectivorous species eating ants treated with a gel. Due to the variety of uses, target organisms and application types, the biocidal environmental risk assessment regularly requires the adaptation or development of new exposure scenarios which complete existing European guidance documents and enhance the environmental risk assessment.

MO278

Exposure of non-target prey and predators to anticoagulant rodenticides

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Anticoagulant rodenticides (ARs) are the main management tool for the reduction of commensal rodents worldwide. However, ARs are toxic to all warm blooded organisms and can lead to wildlife exposure and poisoning. The details of the connection between primary exposure of non-target small mammals by direct AR bait consumption and secondary exposure of predators through uptake of contaminated prey are often unknown. In field studies we assessed the exposure of small mammals and estimated AR exposure risk for barn owls (*Tyto alba*) based on AR residues in small mammals during baiting campaigns on farms and the diet of barn owls in the farm environment. In addition, we screened barn owl spit pellets, liver samples of prey cached by barn owls, and carcasses of predators for AR residues to clarify the exposure pathway from bait to predator. Furthermore, we analyzed local parameters affecting AR exposure in red foxes on regional scale to identify spatial drivers of AR exposure in top predators. AR residue concentration of non-target small mammals was highest within 15 m of bait points and low concentrations were detected in animals caught at a distance of up to 87 m. *Apodemus* species and the shrew *Crocidura russula* regularly carried residues (sometimes at high concentration), whereas exposure in *Microtus* species was low. The exposure risk of barn owls was highest in autumn when owls increasingly preyed on *Apodemus* species and low in summer when owls mainly preyed on *Microtus* species. ARs were rarely found in spit pellets but were present in 13% of prey items collected from nest boxes. About 36% of predatory birds and owls and 60% of red foxes (*Vulpes vulpes*) that were sampled across Germany carried AR residues. In foxes, the presence of AR residues was correlated to the percentage of urban area and livestock density. The results demonstrate that AR exposure of non-targets occur more or less often depending on species and several environmental correlates incl. distance to bait points (small mammals), seasonal prey composition (barn owls), and livestock density (foxes). Knowledge about environmental drivers of non-target exposure to ARs can be used to adjust risk mitigation strategies. (this study was funded by German Federal Environment Agency grant #371063401)

MO279

Resistance of Norway rats (Rattus norvegicus) to anticoagulant rodenticides: Distribution in Germany and factors determining the spread

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Resistance to anticoagulant rodenticides used as biocides is known in the commensal rodent species, *Rattus norvegicus*, *R. rattus* and *Mus musculus*. Knowledge about resistance distribution and factors determining its spread are essential for an effective management with focus on mitigated risks to the environment. The polymorphism Tyr139Cys of the VKORC1-gen is the genetic marker of anticoagulant resistance in *R. norvegicus* in Germany. Effective management of resistant Norway rats is not possible with first-generation anticoagulants (warfarin, chlorphacinone, coumatetralyl) as well as bromadiolone and difenacoum. Nevertheless, because of their high toxicity the active substances flocoumafen, brodifacoum and difethialone should be used only in cases of detected resistance in order to mitigate environmental risks entailed with the use of these compounds. Rat samples have been collected since 1999 in the resistance area in Northwest Germany. On local scale we investigated both, the frequency of the resistance gene by ArmsPCR and the genetic distance using fragment length polymorphisms of nine single sequence repeat markers to determine the spread of Norway rats within the resistance area. We analyzed 189 individuals from two successive years and 13 farms. The analyzed rats showed high site fidelity; places of capture were identically to the place of origin. The total population could be virtually subdivided into eight units with different degree of gene flow. Geographic structure rather than spatial distance was found to structure the rat population. While most farming sites along a small creek were found to be populated by rats of the same clustering unit farms with increasing distance to the creek differed markedly. Three completely isolated clustering units were detected without gene flow to the remaining investigated population. Virtual subdivided units could be correlated to Tyr139Cys allele distribution. While Tyr139Cys was present at all sites it occurred disproportional frequent in the isolated units. In consequence, the applications of the most potent anticoagulants are mandatory to combat resistant infestations. Knowledge about rat migration will allow targeted applications of active substances appropriate to the risk of resistance occurring in order to mitigate environmental risk where the exposure of non-target animals cannot be completely avoided.

Life Cycle Data and Modeling Developments - From Data Collection to Usage (P)

MO280
Life Cycle Assessment of bio-based solvents for paint and coating industries
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Keywords: Solvents, Bio-based products, Environmental impacts, Sustainability
The global consumption of solvents is significant, with almost 28 million metric tons consumed in 2012 (Linak and Bizzari, 2013). The paints and coatings sectors are the main solvent consumers. This demand is predicted to increase by 2.9 % per year until 2019 (Ceresana, 2014). Nowadays, the solvents used in the paint and coating industries are currently produced from petrochemical sources. In the context of sustainable development, the replacement of traditional solvents by new green alternatives has to be studied. The ECOBIOFOR project, developed by a consortium of 11 European partners from 6 different countries, will help the transition of European paints and coatings industry from petrochemicals to bio-based products. The main objective of the ECOBIOFOR project is to develop novel bio-based solvents from renewable building blocks thanks to easier chemical or biotech transformations processes. In parallel to the syntheses development, a Life Cycle Assessment (LCA) of these new bio-based transformation processes is considered, using the ILCD method with some improvements, in order to assess their potential environmental impacts and so select the best-developed options. These new pathways are also compared to traditional petrochemical processes. After we defined the goal and scope of this LCA study, difficulties have been encountered on the data collection due to confidentiality issues and lack of data, especially for the chemicals in the Ecoinvent database. Methods to overcome these difficulties have thus to be developed from the point of view of the foreground and background data. Therefore, data could be obtained either from specific collection (directly from the manufacturers or in the R&D laboratory), by using proxy to model the considered molecule by a similar one already available in the databases or finally by semi-specific modelling, *ie*. reconstructing the synthesis pathway through retrosynthesis to obtain building blocks available in Ecoinvent v3. **References :**
Ceresana (2014). Market Study: Solvents. Linak, E., and Bizzari, S. (2013). Global Solvents: Opportunities for Greener Solvents (IHS Chemical)

MO281
Conversion of international environmental reporting data to LCA data for SCP
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Consumption patterns have shown to increase the use of natural resources for the production of goods and services. A national project has been set up to provide support to Swedish authorities (notably the Swedish Environmental Protection Agency and the Swedish Agency for Marine and Water Management) to gain a better understanding of the reasons for why some environmental problems are difficult to forecast - thereby making them difficult to mitigate by conventional methods - with a special focus linked to Sustainable Consumption and Production (SCP). The theoretical parts, which cover a major portion of the overall work, include a methodological development on a scientific basis where long-term national monitoring data provided by the Consortium for Environmental Data reporting in Sweden (SMED), in various contexts such as the Industry Emissions Directive (IED), the UNFCCC, and others, are subject to assessments on how such data can be converted into LCA-based information. Such an assessment will primarily include two essential aspects ♀_ one dealing with a number of technical considerations for allocation and other LCA aspects, the other with non-technical aspects, e.g. the proper use of confidential data. This part of the project will also explore how SMED data could be used for trend analysis enabling identification of key pollutant emission sources as an input to a deeper understanding of why some environmental quality objectives, specifically those related to SCP, are hard to fulfil. The practical and operational parts will focus on synthesis and implementation of results, in which conclusions are drawn on how the SMED work can be better linked to the work related to the Swedish environmental goals, as well as providing some perspectives for suggesting additional needs of legislative and other means for better control of SCP. This part will also focus on international outreach, in which the key focus will be to provide a future input to the UN work on establishing a "Global Network for Interoperable LCA Data Bases (GlobaLCAccess)", and link these activities to European and international standardisation work.

MO282
LCI data sets for up-stream processes of fossil energy for Germany today and 2030
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The German "Energiewende" (Eng. energy transition) aims at enhancing the share of renewable energy carriers in order to mitigate Green House Gas emissions. Mitigation is calculated against the reference of fossil energy sources such as crude oil and natural gas. However, environmental impacts of extraction of fossil energy carries also change in the course of time due to technological performance but also due to an increasing use of unconventional sources. For a realistic estimate of national GHG balances in different energy scenarios, data for up-stream processes are most crucial: Whereas the environmental impacts of the combustion technologies mostly are well-known, the extraction of fossil fuels receives little attention in life cycle assessment (LCA) studies (Bouman *et al*., 2015). This translates into the lack of data availability showing changes of conventional and unconventional extraction technologies in LCA databases. The performance of high quality life cycle assessments requires life cycle inventory data representing the current state of technology. To this end, this study analyses environmental impacts of current production technologies of petroleum and natural gas. Life cycle inventory datasets describing the current production of crude oil and natural gas in various regions are provided and compared with older datasets produced in 2000. The production regions examined are those which provide Germany with crude oil or natural gas. Furthermore, in order to analyse the future global warming potential (GWP) regarding the production of 1 kg crude oil and 1m3 natural gas for usage in Germany, scenarios for 2030 are developed. The LCAs are generated according to ISO 14040 and ISO 14044. For the bulk of oil producing regions, a decrease of GWP compared to 2000 is identified. Simultaneously, the GWP of natural gas production has increased in all examined regions of production. Based on the scenarios, future GWP of natural gas production for Germany will be higher than today. The results of the scenarios for the crude oil production are less distinct. The results range from nearly constant GWP to increased GWP. Taking into account the current trend for decreasing GHG emissions during oil production, even a sinking GWP might be possible.

MO283
Assessing the environmental improvement of new technologies in the detection of pathogens in drinking water - the wider impact of the AQUAVALENS project
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Life Cycle Assessment can be applied to a wide range of case studies considering that it can be seen as a philosophy for undertaking the countable procedure of all pollutant exchanges between the system analyzed and the environment. This system can take the form of a variety of activities, services and products, from an entire industrial sector of a country to any particular action that a person can perform. The LCA practitioner must face the challenges posed by the peculiarities of each system, adapting the assessment to the demands on data availability, uncertainty of the input data, resolution

of the model, necessary assumptions, sensitivity to unsettled parameters, etc. In the presented study we analyze the consequences of adopting decisions on the mentioned features in the design of the LCA models used. The systems to be evaluated are the novel platforms developed under the project AQUAVALENS for the detection of pathogens in drinking water. The aim of the project is to enable the water system managers, food growers and manufacturers to better control the safety of their water supplies by using newfangled technologies that integrate sample preparation and detection into a single platform. The Carbon Footprint calculation of these platforms will be used to determine their sustainability as part of final objectives of the project, which include the protection of human health, improving the effectiveness of Water Safety Plans, adaptation to climate change and control of outbreaks of infectious diseases. The challenges of developing a LCA of the systems under study rely on the difficulty for setting a high number of parameters related, on the one hand, with the platform manufacturing and , on the other hand, with the protocols and procedures to use them. The complexities have their origin in the diversity of platforms, manufacturers and potential users, which make valid the saying that there is more than one way to skin a cat. This variety have forced us to perform the study in a iterative way, where questionnaires and model drafts have consecutive introduce the needed input data from the most general to the smallest detail so that each model was refined to obtain a degree of uncertainty consistent with the objectives of the study. Particularly, the decisions and assumptions made were based on robust uncertainty and sensitivity analyses.

MO284
Prospective performance indicators of electricity production in Spain
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Although environmental modelling methodologies such as Life Cycle Assessment (LCA) are well-known tools for the analysis of sustainability issues, there is a gap in managing the time dimension. Many studies carry out assessments assuming predefined systems concerning the future or use roadmaps from the literature. This work involves a detailed LCA study from a consequential perspective by using a national energy systems optimisation model, TIMES-Spain. This methodological linkage strengthens the discussion on how the electricity production mixes should be built in the future according to key prospective performance indicators. TIMES-Spain, built with the TIMES energy optimisation model generator developed by ETSAP Implementing Agreement of the International Energy Agency, provides energy-related technology mixes for satisfying the demand of energy services in all economic sectors (industry, transport, etc.) under different assumptions. A couple of scenarios were implemented: a Business as Usual scenario “BaU” with restrictions from European Directives, and an exploratory scenario “80%” enforcing an 80% reduction in the total Spanish CO_2 emissions by 2050 with respect to 2005 levels. The evolution of the electricity production mixes from the optimisation process shows that the scenario “BaU” results in a major presence of natural gas cogeneration plants in the long term, whereas the scenario “80%” prioritises wind and biomass options. LCA computation was performed for the set of existing and new technologies. Besides, a LCA/TIMES harmonisation procedure was carried out avoiding a potential double counting of emissions. In this regard, existing processes in TIMES-Spain were adapted to include a fraction of the burdens linked to infrastructure by assuming a residual lifetime according to the year of installation. The evolution of ten life-cycle indicators (from ILCD 2011 and ReCiPe methodologies) shows global reductions in the long term ranging from 21% for Ozone Depletion to 85% for Acidification in the scenario “BaU”, and reaching up to 87% for Acidification in the scenario “80%”. Nonetheless, Abiotic Depletion grows significantly due to the rare metals required in the production of solar cells, highly present in the future mix. Energy systems modelling is found to be a valuable tool to perform prospective LCA studies in the field of energy, adding the time dimension to the sustainability assessment.

MO285
Lessons Learned in Developing an Environmental Product Declaration Program for the Asphalt Industry in North America
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The objective of this presentation is to report the technical and organizational challenges involved in the development of the North American Environmental Product Declaration program for asphalt mixtures. It explores how differences in stakeholder priorities and perspectives, in the pavement construction industry, directly shape the Product Category Rules (PCR) defining the program. The primary challenge identified is how to ensure technical rigor of the underlying LCA, while recognizing the interests of the stakeholders and ensuring the delivery of a program that is effective. The presentation discusses how technical issues regarding system boundary choice, data use and allocation presented challenges for the PCR Development Working Group accounting for different stakeholder interests. Insights into the discussions that informed the PCR development are provided.

MO286
LCAnet.de - A new network for LCA practitioners in Germany
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LCA networks provide contacts and allow for exchange of knowledge and questions within the LCA community. They can be an extremely helpful address for solving problems and obtaining information about recent developments, new publications and upcoming workshops, especially for new practitioners. While there are several important LCA networks on international level or in other regions, there is little local activity in Germany. LCAnet.de is a recently founded non-profit organization dedicated to the exchange of knowledge among – especially young – LCA practitioners. It aims at building up a decentralized structure with several regional nodes in Germany. This should keep distances between the actors short, allowing for direct communication and shorter, cheaper and environmental friendly travels to workshops. LCAnet.de's first node is in the city of Karlsruhe reaching from Freiburg in the south to Darmstadt in the north. LCAnet.de is an integrative network, open for everyone who is working in a LCA context within the network's regions. Therefore the language of communication is English. By distributing organizational tasks among the network's members, LCAnet.de can run on a small budget. Consequently member fees are very low, and participation in workshops is either free or inexpensive. This eases the access for people who are not able to pay considerable member fees such as students, PhD-candidates or professionals who are hardly connected to the academic LCA community. LCAnet.de provides a web page with relevant news and a forum for addressing specific questions to the community. The core activity of LCAnet.de is the organization of regional workshops at least twice per year. Each workshop addresses a single, LCA-related topic, aiming at learning, discussing, problem solving, method developing and networking. This poster presents the non-profit organization LCAnet.de, provides information about its principal goals, how to become member and about the services and networking activities provided by the network. It invites especially young, but also experienced LCA practitioners to join and to actively contribute to the development of this initiative.

MO287
Ecospold 02 - ILCD: mapping and format converter
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In a recently completed project carried out for the Joint Research Centre (JRC) of the European Commission, GreenDelta updated the International reference Life Cycle Data system (ILCD) reference elementary flow list. Basing on the existing mapping file available in GreenDelta’s open LCA format converter, the ILCD flowlist has been revised and updated, in collaboration with JRC, in order to cover all the elementary flows available in EcoSpold02 nomenclature. For the EcoSpold02 flows not mappable in the ILCD reference flow list, new flows have been added to the ILCD lsit. Almost 400 new flows have been created, and more than 3400 have been mapped, and the nomenclature has been further investigated also accounting for the new needs of the upcoming Environmental Footprint (PEF and OEF) scheme, which is now in the pilot phase, under the supervision of DG Environment. The project has been funded by JRC within the institutional activities, therefore the converter was developed only for the conversion from Ecospold02 to ILCD and not vice-versa, since the other formats and nomenclature are not officially adopted or endorsed in EU Policy. However, the results of the project are now available also in the framework of the international cooperation for the Global Network of interoperable LCA databases, led by UNEP, where the aim is to expand the mapping also to other format and nomenclature systems, and to enhance the converter in order to allow multi-directional convertibility.

Improving the usability of ecotoxicology in regulatory decision-making: findings from a SETAC Pellston® Workshop (P)

MO288
Updating Environmental Quality Standards using REACH disseminated data: an illustration of transfer of knowledge between policies
G. Deviller, CJExpertPOPs
Article 16 of the Water Framework Directive (WFD, EC 2000) sets out the strategy against chemical pollution of surface waterbodies. The chemical status assessment is used alongside the ecological status assessment to determine the overall quality of a waterbody. The Environmental Quality Standards (EQSs) Directive (EC 2008a) establishes concentration limits for 33 Priority Substances and 8 other pollutants. EQSs were derived for the Priority Substances at a European level and are used to assess the chemical status of water bodies in all Member States. In addition, the WFD establishes the principles to be applied by

the Member States to develop EQSs for Specific Pollutants that are 'discharged in significant quantities'. Compliance with EQSs for Specific Pollutants forms part of the assessment of ecological status. Derivation of EQSs is based on (eco)toxicological data on organisms that are relevant for the identified protection goal: the pelagic organisms for waters (fresh and marine), the benthic organisms for sediments (fresh and marine) and mammals/birds for secondary poisoning. Many of the EQS that have been derived up to now have used peer-reviewed data and in many cases data gaps were observed for one or more compartments. However, it is recognised that the risk assessment paradigm on which the EQS derivation is based could lead to unworkable and/or unrealistically low EQS values in case of data lacking (Technical Guidance for deriving EQSs, 2011). With REACH entering into force, many studies have been performed by industry in order to comply with their regulatory obligations. The dissemination of data of registered dossiers on ECHA's publicly available website has given considerable opportunities to improve our knowledge of the substances properties and to reassess previous work like EQS derivation. This presentation will show how REACH disseminated data have been assessed for EQS derivation purposes (relevance and reliability) and if these new data could decrease the uncertainties attached to the existing EQS and finally improve the global assessment of the chemical status of European waters.

Innovative techniques for monitoring chemicals in the environment (P)

TU001

THE IMPACT OF β-BLOCKERS ON THE SOIL MICROBIAL PHYSIOLOGICAL DIVERSITY

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Pharmaceuticals and their metabolites reach the terrestrial ecosystems through the application of wastewater and contaminated biosolids to agricultural soils. There, these products may impact the microbial community. We have evaluated the effect of six widely consumed β-blockers on soil microbial communities from an ecological farming crop field using the community-level physiological profile –CLPP-. This method is based on the rate of carbon substrate degradation on microplates (Biolog Ecoplates®). The impact of the pharmaceuticals has been assessed by comparing the Averaged Well Color Development –AWCD- of each treatmente, as an integrative indicator of the physiological ability of the microbial community for degrading all carbon sources. Nadolol provoked a slight decrease in the AWCD compared to control. On the other hand, a stimulatory effect was observed for the other β-blockers. The Shannon diversity índex (H) was used to evaluate the physiological diversity of the microbial communities. The soils treated with propranolol ($p< 0,05$) and metoprolol ($p< 0,01$) presented a significantly lower physiological diversity. The β-blockers also significantly decreased the degradation of polymer carbon and carboxylic and ketonic acids sources and significantly increased the amines/amides. No differences were detected in the use of carbohydrates and amino acids, except for the atenolol that showed a significant increase ($p< 0,05$) in the use of amino acids. Results shown that pharmaceuticals on soil microbial communities, at environmentally relevant concentration (around 100 mg/Kg), altered key ecosystem functions as that of the carbon cicle.

TU002

Eco-toxicity and human toxicity impact in urban area: case of accumulated trace metal air pollution analysis on cemetery mosses in Paris city

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The monitoring of global atmospheric pollution in urban and peri-urban areas is in a crucial phase where expectations of society in terms of environmental issues often exceed the capacity of integrative measures to track pollutants in the long term. Mosses are useful ubiquitous accumulation bio-monitors and as such were used for European surveys. Furthermore, anthropogenic emissions (e.g., heavy metals) being closely related to ecosystem and human health, integrative risk assessment is also of a major interest in citizens' expectations. In this study, we tested the hypothesis that cemeteries are appropriate sampling sites to evaluate moss accumulation of air pollutants in urban areas, and that the USEtox impact assessment model is useful to provide relevant eco-toxicity and human toxicity impacts. For this purpose, we sampled mosses growing on gravestones in 21 urban and peri-urban cemeteries in the Parisian metropolitan area. We focused on *Grimmia pulvinata*, a species abundantly found in all studied cemeteries. We quantified 19 elements with TXRF-SSM method. For eco-toxicity and human toxicity impact and mapping, the use of USEtox method of life cycle impact assessment and its characterization factors allowed to characterize factors and categories (human toxicity and ecotoxicity) related to trace metals in air emissions. We show that enrichment factors for some markers of road traffic (e.g. Pb, V, Zn, Cr) indicate that the highest polluted cemeteries were located near the highly frequented ring road of Paris and under the influence of prevailing winds coming from the Southwest. The sites with the lowest pollution appeared in peri-urban cemeteries, adjoining forest or farming landscapes, but also in large and relatively woody cemeteries located in the centre of Paris. The calculation and mapping of the accumulated eco-toxicity and human toxicity impacts allowed showing the distribution of highest and lowest polluted areas in complex urban fabric as Paris area. Observed differences between environmental and human toxicity distributions show that we can expect more spatial variability of toxicity impact even in densely urbanized areas that usually thought.

TU003

E-Board - a new in situ method for assessing the reproduction-related traits of chironomids: proof of concept and potential perspectives of use

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Further progress in the development of reliable biomonitoring strategies requires to better link effects in aquatic ecological systems to ambient contaminations. Among existing tools, *in situ* bioassays using caging method represent an interesting way to achieve this challenge. However, elaboration of adapted exposure chambers and suitable operating procedures is still required, particularly to assess ecological relevant traits such as those related to the reproduction. In such context, we developed a new device (Emergence board - E-Board) which allows assessing in rivers the development of the *Chironomus riparius* species from the early fourth instar larvae to the adult stage. The system is based on the hypothesis that chironomid larvae mainly feed on freshly deposited suspended matters that represent the major exposure route to contaminants for them. The system acts as a suspended matter trap floating in the subsurface of the water equipped of an emergence trap for catching chironomid adults. The system was tested in actual field conditions. Its easy handling allowed obtaining data which demonstrated its applicability for assessing the development of the chironomids. Moreover, by adapting energy-based models specifically developed in the laboratory for the species *C. riparius*, we were able to predict the growth pattern and the emergence of chironomids in real environmental conditions. The method offer the possibility to follow ecologically relevant responses on sensitive life stages or periods for individuals which are difficult to take into consideration in conventional community surveys and which are crucial to infer impacts at population level. The E-Board represents thus a promising new in situ tool in perspective of evaluation of the quality of the ecosystems.

TU004

Development of sensor based on optical fibre for identification of chemical parameters in surface water

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Permanent monitoring of contaminants that can disturb the quality of surface water is required, due to increased environmental pollution from anthropogenic sources. Standard laboratory methods revealed certain disadvantages relating to the high cost, complexity of analysis and the possibility of losing the desired analyte caused by human error in sampling, storage and transport of samples. Fiber optic sensors (FOS) are convenient technology for monitoring of surface and ground water and to provide continuous and real data about the current state of water bodies. FOS have certain advantages over conventional methods such as: simple design, small size, possible use in hazardous chemical environments that can damage the measuring equipment, can be used for remote sensing and in distant areas, safety because of the low optical power and the absence of electric current at the sensing point and resistance corrosion. This paper presents a prototype of colour FOS for detection of different inorganic parameters in surface water. Implemented sensor converts the RGB color model to HSV color model and based on the color intensity of the sample determines the concentration for parameters of interest. The concentration of the chemical parameters is calculated by introducing the obtained values of H (Hue), V (Value), S (Saturation) in transfer function. Colour FOS based on HSV model was designed for the determination oforthophosphate, nitrite, sulfate, $Cr^{6+}_{(aq)}$ and total chlorine in surface water samples. Results obtained with the applied sensor are compared to the results obtained with laboratory analysis to prove the efficiency of the device. FOS has proven to be very suitable for use in the laboratory under controlled conditions as low-cost solution to replace expensive standard equipment. Future research should be focused on performance improvement and on increasing the sensitivity of the device, which will be achieved by choosing better quality LEDs with a wider wavelengths range. Keywords: fiber optic sensors, low-cost measurements, surface water Acknowledgement: The research has been supported by Ministry of Education, Science and Technological Development of Republic of Serbia under project Development of methods, sensors and systems for monitoring quality of water, air and soil, III43008.

TU005

FROM TOXICOLOGICAL BIOASSAY AND CHEMOMETRICS ANALYSIS TO A RAMAN SPECTROSCOPY MULTIPARAMETRIC BIOSENSOR

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In the field of toxicological bioassays, the toxicity assessment of a pollutant or a mixture on living organisms is often based on the observation of a single physiological parameter (e.g.: bioluminescence inhibition, cellular death, enzymatic activity). The monoparametric aspect of such bioassays limits their performance in terms of specificity and sensitivity. They need to be associated in order to provide a global toxicity measurement, and lead to the multiplication of the implemented tests. In this context, the latest progresses in the Raman spectroscopy open new research perspectives on fast method of observing metabolic responses against toxic agents. Indeed, each spectrum is made of more than 2700 data points which represent a molecular fingerprint of the observed samples. This multiparametric approach provides an overview of the physiological changes caused by the pollutants. Thus, the comparison of spectra from bacteria exposed or not, is used to define a “signature” for a given toxic effect and overcome the limitations of other biosensor techniques. Moreover, the physiological spectral fingerprint is both rich and complex. Consequently, to be properly analyzed it needs complex chemometrics methods. In this study, statistical multivariate analyses have been implemented to highlight the differences between spectra of the bacteria *Escherichia coli* exposed to various increasing arsenic concentrations. The results show both a dose-response and an exposure time effect of the arsenic on *E. coli* cells. Furthermore, the fine analysis of the Raman spectroscopy fingerprints permits to identify the cellular macromolecules impacted by this chemical. The highlighted changes are in accordance with the expected toxic effects. In the future, a toxic signature of pollutants and a more precise overview of their metabolic target might thus be identified by the Raman multiparametric observations of toxic effects on microorganisms’ metabolism coupled to chemometrics tools.

TU006

Application of LC-APCI-qTOF-MS for simultaneous analysis of short-, medium- and long-chain chlorinated paraffins in technical and environmental samples

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Chlorinated paraffins (CP) are high production volume chemicals (1 Mio t/a in 2009) used as extreme pressure additives, plasticisers and flame retardants. They are of concern due to their persistence, bioaccumulation potential and the limited information on their environmental fate and toxicity. Analysis of CPs in environmental samples is a nightmare due to the large number of congeners and isomers (chain length: C_{10}-C_{28}, Cl-degree: 30-70%) and the lack of suitable standards. Recent analytical methods mainly focus on short-chain CPs (SCCPs). SCCPs are currently discussed to be classified as persistent organic pollutant by the Stockholm Convention Review Committee. Thus, a shift in production and emission to medium- and long-chain CPs (MCCPs and LCCPs) is expected, requiring respective analytical methods. Most studies on CPs apply gas chromatography (GC) with mass spectrometry (MS). However, GC is not suitable for analysing CPs with increased chain length due to their low volatility. Recently, Bogdal et al. 2015 (Anal. Chem. 87, 2852-2860) developed a method overcoming the need for separation using direct liquid injection and focussing on accurate mass determination. They apply atmospheric pressure chemical ionisation (APCI) under chlorine-enhanced conditions in negative ion mode followed by quadrupole time-of-flight (qTOF) MS. Their method allows rapid (< 1min) and simultaneous analysis of SCCP, MCCP and LCCP, featuring increasing sensitivity with increasing chain length. The present study investigates how different CP congeners compete for the available chloride. Liquid chromatography (LC) was applied to partially separate CPs. The developed LC-method was able to overcome drawbacks of the direct injection such as matrix interferences and co-elution of analytes with similar masses. The optimised method has been applied to technical and environmental samples and compared to direct injection. Homologue-specific patterns were validated using a carbon skeleton method which relies on the complete hydrodechlorination of CPs to respective alkanes. Corresponding results will be presented. This LC method is the first chromatographic method for simultaneous analysis of SCCP, MCCP and LCCPs since 2004 and it is the first LC method based on high-resolution MS for CP analysis applied to environmental sample.

TU007

Monitoring of pesticides in air - sampling technique implications

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Pesticides applied on agricultural fields can enter the atmospheric compartment due to volatilization. Depending on e.g. compound intrinsic properties and climatic conditions pesticides can thus be subjected to short- or long-range atmospheric transportation following application. Monitoring studies frequently demonstrate occurrence of currently used pesticides in the atmosphere, including trans-boundary transport. However, less is known on the importance of the collection procedure of air-born pesticides in order to enhance interpretation of the transport processes involved. Air samples were collected during 2010 – 2012 in a forested area in the south of Sweden, with little agricultural activities in the vicinity. Using high-volume sampling air was pumped, during 5-7 days, through a glass-fiber filter followed by polyurethane foam (PUF) and a hydrophobic crosslinked polystyrene copolymer resin (XAD), in a PUF-XAD-PUF-sandwich for simultaneous sampling of both particle bound and gas phase pesticides. The filter and the three adsorbents were Soxtec extracted separately to investigate the distribution of pesticides. The samples (n = 34) were analysed for 67 pesticides on GC-MS. Overall, a total of 43 pesticides were detected in the air samples. The most frequently detected (100% of the samples) were lindane, α-HCH, HCB, α-chlordane and γ-chlordane, i.e. pesticides banned since more than a decade within the EU. The pesticide found in the highest concentration was prosulfocarb at 13 ng/m^3. On the filter 25 pesticides were identified, most of them currently being used in Sweden. Some of these were only detected on the filter. The first PUF contained 81% of the total pesticide concentration and out of the 33 pesticides detected 19 of them were not approved for use in EU. The XAD and the second PUF generally contained low amounts of a few substances also found in the first PUF. Of all 43 detected pesticides only dichlobenil, α-HCH, heptachlor, HCB and trifluralin gave a breakthrough of more than 30% from the first PUF to the XAD and second PUF. The XAD was essential for capturing a few very volatile, obsolete pesticides, but generally the first PUF was efficient enough to adsorb pesticides in gas phase at environmental relevant concentration levels. For an accurate estimate of the pesticide concentrations in air, especially for currently used pesticides, it is vital to include the particulate phase by adding a filter material in the collection procedure.

TU008

Spatio - temporal quantification of gadolinium and iodide complex uptake in zebra fish embryo

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Key parameters for adverse effects of chemicals to organisms are chemical uptake by and distribution within the organism leading to the “internal exposure” as pivotal factor for biological effects of chemicals. Recently the zebra fish embryo (ZFE) has been suggested as a model organism to observe potential toxicological effects of anthropogenic compounds. In order to correlate toxicological effects in the ZFE with uptake and distribution data analytical methods have to be provided. In future such correlations are required as input for toxicokinetic/toxicodynamic models which will be able to predict effects of chemicals on ZFE. These predictions are an important component of risk assessment approaches. Quantification of uptake based on internal concentration analysis can be regarded as state-of-the-art. Compounds such as gadolinium and iodide complexes used as contrast agents were selected as examples to study the uptake and the distribution in the ZFE because of their increasing application. This work aims at developing analytical methods to provide internal concentration data as well as compound distribution data in ZFE by means of laser ablation hyphenated to inductively coupled plasma mass spectrometry (LA-ICP-MS). Exposure studies with ZFE and gadolinium and iodide complexes are presented. Internal concentration and compound distribution in ZFE were determined after 96 h of exposure. Internal element concentrations were quantified by ICP-MS. LA hyphenated to ICP-MS was applied to map gadolinium and iodide distribution in cross-section of ZFE. Calibration of LA-ICPMS instrumentation was done by agarose standards with known gadolinium and iodide concentration. Exposure studies with iodide compounds (iohexol, iomeprol, iopromide, iopamidol, amidotrizoic acid) revealed a compound uptake between 10 and 18% of the external concentration. Uptake of the investigated gadolinium complexes (Gd-Dota, Gd-Dtba, and Gd-EOB-Dtba) was in the range between 1 and 3% of the external concentration. Qualitative spatial resolved analysis indicated elevated iodide concentrations in the ZFE cross-sections. For both, iodide and gadolinium complexes quantitative distribution data will be discussed. The presented method provides a significant progress towards quantitative data which is required for process understanding and risk assessment. Additionally the analytical methodologies presented here can be transferred to other elements which are accessible to ICP-MS detection.

TU009

Determination of short-chain and medium-chain chlorinated paraffins in fish tissues by gas chromatography coupled to high-resolution time-of flight mass spectrometry

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Short-chain chlorinated paraffins (SCCPs) and medium-chain chlorinated paraffins (MCCPs) are chlorinated alkanes of variable chain length and chlorination degree. They constitute a complex mixture of straight and branched compounds (>10,000), used in a wide range of applications as lubricants in metal working or in the leather industry, plasticizers or flame retardants as well as in sealants, paints and coatings. Both SCCPs and MCCPs are considered to fulfill the PBT criteria (persistent, bioaccumulative and toxic chemicals) since they exhibit high bioaccumulation factors in fish as well as elevated acute toxicity towards aquatic biota. As a matter of fact, SCCPs have been proposed for listing under the Stockholm Convention on Persistent Organic Pollutants. Although Low Resolution Mass Spectrometry (LRMS) operated in Electron Capture Negative Chemical Ionization (ECNI) is frequently used for the determination of SCCPs and MCCPs, the preferred analytical method is High Resolution Mass Spectrometry (HRMS), usually performed with a magnetic sector analyzer. This technique minimizes interferences from other contaminants and between SCCPs and MCCPs; it also enables the quantification of specific homologue groups (i.e. identical chain length and number of chlorine atoms). Over the years, High Resolution Time of Flight (ToF) and Orbitrap MS technologies have become available in numerous laboratories; these techniques, however, have not been fully explored for the determination of SCCPs and MCCPs in environmental samples. Thus, the aim of the present work was to develop a simple method for the determination of SCCPs and MCCPs in fish tissues, based on a single-step adsorption chromatography clean-up followed by analysis using gas chromatography coupled to ToF MS. The validated and optimized method was applied to the determination of SCCPs and MCCPs in the fillets of selected fishes from the Rhône River basin (South Eastern France). The target species was the Common barbel (*Barbus barbus*), a typical benthic feeder widely used for biomonitoring purposes in the European Union. *Acknowledgements* - This study was undertaken with the financial support of ONEMA, in the frame of the Investments for the future Programme (Cluster of Excellence COTE, ANR-10-LABX-45). CPER A2E (Aquitaine region and FEDER (Europe is moving in Aquitaine with the European Regional Development Fund (FEDER)) is acknowledged for financial support.

TU010

Performance of polyethylene passive samplers to assess DDx bioaccumulation in freshwater mussels

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Contaminants in the aqueous environment are present as dissolved, colloid-bound, and sediment-associated forms. The distribution of contaminants between these phases is a central issue as it strongly influences the sediment and water quality. In the past, sediment quality criteria were set using a simple equilibrium partitioning (EqP) approach seeking to relate dissolved and sorbed contaminants. However, this approach does not account for different aspects such as colloid speciation and specific sorption effects, that may dramatically change environmental impacts. Hence, in the last decades great attention was focused on new methods that can allow us to account for these issues when defining the quality of a sediment. Among these, the use of passive sampling is becoming an attractive option, as this method allows direct estimation of the pore water concentration of a chemical, i.e. a metric that reveals the bio-available fraction. In this work, we used passive samplers to assess the bioavailable forms of DDx in sediments collected from a lake located in northern Italy; the results were compared with those obtained by exposing the freshwater mussels, *Unio pictorum*, to the same sediments. Low density polyethylene devices (PEDs) and organisms were exposed in glass jars containing the sediment collected from different sampling stations of the lake for either 10 or 28 days. At the end of each test, the PEDs and mussels were analyzed for DDx concentrations. The concentrations obtained in the PEDs were then used to estimate the tissue concentrations in the corresponding exposed mussels. The results showed that the PED-deduced values were consistent with the measured levels for the 28-days tests, whereas the tissue concentrations predicted from passive samplers data were in some cases higher than the ones measured in the organism tissue after 10 days of exposure. This suggests that approach to equilibration of the sediment with a PED was faster than uptake by the benthic organism. These results show that PE samplers data may be quite effectively used to predict the concentration of a contaminant expected in the tissue of freshwater mussels, such as *U. pictorum*, thus allowing investigators to predict the bioavailabile fraction of organic contaminants in an easier and reliable way.

TU011

Perfluoroalkyl substances in aquatic environment - comparison of fish and passive sampling approaches

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The concentrations of seven perfluoroalkyl substances (PFASs) were investigated in 36 European chub (*Squalius cephalus*) individuals from six localities in the Czech Republic. Chub muscle and liver tissue were analysed at all sampling sites. In addition, analyses of 16 target PFASs were performed in Polar Organic Chemical Integrative Samplers (POCISs) deployed in the water at the same sampling sites. We evaluated the possibility of using passive samplers as a standardized method for monitoring PFAS contamination in aquatic environments and the mutual relationships between determined concentrations. Only perfluorooctane sulphonate was above the LOQ in fish muscle samples and 52% of the analysed fish individuals exceeded the Environmental Quality Standard for water biota. Fish muscle concentration is also particularly important for risk assessment of fish consumers. The comparison of fish tissue results with published data showed the similarity of the Czech results with those found in Germany and France. However, fish liver analysis and the passive sampling approach resulted in different fish exposure scenarios. The total concentration of PFASs in fish liver tissue was strongly correlated with POCIS data, but pollutant patterns differed between these two matrices. The differences could be attributed to the metabolic activity of the living organism. In addition to providing a different view regarding the real PFAS cocktail to which the fish are exposed, POCISs fulfil the Three Rs strategy (replacement, reduction, and refinement) in animal testing.

Biological effects of emerging micro pollutants at realistic environmental concentrations (P)

TU012

A Preliminary Assessment of the Future Needs for Micropollutants in Turkey under Water Framework Directive Using Bibliometric Approach

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Industrial chemicals, pesticides, drugs and drug residuals (pharmaceuticals), personal care products, steroid hormones and many other emerging pollutants are being considered under the term "micropollutants". Due to the diversity of micropollutants, the low concentrations, low treatment efficiency of micropollutants and their toxic effects, it is significant to monitor the micropollutants both in the environmental media and in the humans. To date, although discharge guidelines and standards do not exist for most micropollutants, some European Union (EU) countries have adopted regulations to set limit values. In Turkey, "Surface Water Quality Management Directive" adopted from EU-Water Framework Directive, targets to determine the environmental quality standards to regulate, detect and monitor the inorganic and organic micropollutants in surface, coastal and transitional waters, and in discharged wastewaters which may disrupt the water quality and ecological properties of those water bodies. For assessment of micropollutants, quantitative data are required. In order to evaluate the existing literature data and research trends in Turkey on micropollutants, a bibliometric analysis was performed for the period

of 1990 - 2015. Web of Science was used as the main database. The focus was set on selected micropollutants (policyclic aromatic hydrocarbons (PAH), polychlorinated biphenyl (PCB), and bisphenol A (BPA)) and environmental media (water, waste water, and sea water). The results of the study would provide an information on the currents status of the micropollutant species largely found in industrial, agricultural, natural or everyday life, would supply input for both national and international obligations, and would provide contribution to the development of our country-specific quality standards on many micropollutants in the near future. The authors would like to acknowledge the financial support from TUBITAK (Project No: 115Y309).

TU013
Ecotoxicological monitoring of photosystem II inhibitors in 5 small streams in Switzerland
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Small streams represent a major part of the Swiss river network, but so far information about pesticide pollution and pollution dynamics is limited. To investigate the situation in such small rivers, a special monitoring program (SPEZ) was conducted on five streams as part of the National Surface Water Quality Monitoring Network (NAWA) in spring and summer 2015. The aim of this project was to obtain knowledge about peak and baseline pesticide concentrations of over 250 different pesticides as well as their ecotoxicological effects. Selected sampling sites were located in areas intensively used for agriculture with different types of crops (grain, vegetables, vineyards and orchards). 12 h composite water samples were taken continuously from April to August 2015 using automated sampling devices. Samples were extracted using solid phase extraction and subsequently examined for 250 pesticides and metabolites using HPLC-HRMS. To complement the chemical analysis with ecotoxicological effect data, one week composite samples were analyzed for effects on algal photosynthesis and growth using the combined algae assay. This assay is especially sensitive for photosystem II-inhibiting substances. Toxic effects were expressed as diuron equivalent concentrations (DEQ) for photosystem II inhibition and as baseline toxic equivalent concentrations (baseline-TEQ) for growth inhibition. Photosynthesis inhibition was observed in 100% of the water samples, but at different levels. Calculated DEQs ranged between ca. 5 and 300 ng/L. Highest DEQs were found in June in areas with vineyards. In some cases high DEQs could be linked with rain events. During the sampling campaign longer periods without rain occurred whilst DEQs remained elevated. Consequently herbicides were introduced constantly to the streams even without rain events. The ecotoxicological effect data will be compared to use patterns and chemical concentrations measured.

TU014
Embryotoxic effects of reclaimed water in three different freshwater organisms: the Oryzias latipes fish, the Xenopus laevis amphibian and the Physa acuta snail.
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Wastewater reuse is a promising strategy to supply water demands in both water scarcity and water pollution scenarios around the world. The challenge is to develop reliable technical and scientific tools to ensure safety uses of reclaimed water. Although many efforts have been focused on the improvement of technologies for wastewater treatments, the study of environmental impacts produced by wastewater reuse has received less attention. No drinkable reclaimed water uses include farming uses, such as agricultural irrigation and aquaculture. Reuse could also include landscape irrigation, building or replenishing wetlands that support wildlife, creating recreational lakes and several public uses as urban cleaning and park watering. Ecotoxicological studies with freshwater organisms are justified because reclaimed water contains complex mixtures of micro pollutants that can ultimately reach surface water. A comparative analysis of the response of early life stages from different species belonging to three taxa after exposure to reclaimed water is presented. Reclaimed water from wastewater treatment plants (WWTPs) that collect both municipal and municipal/industrial influents with different tertiary treatments (membrane bioreactor/ultraviolet, sand filtration/ultraviolet and lagoons) was sampled twice in one year. A comprehensive hazard assessment was performed by combining chemical analysis and ecotoxicity testing. Samples were analysed to look for 44 emerging contaminants including pharmaceuticals, endocrine disrupters and personal care products (PPCPs). Whole and diluted reclaimed water embriotoxicity was tested in samples and their fortifications obtained by adding a mixture of methylparaben (9 μg/L), PFOS (90 μg/L) and fluoxetine (400 μg/L). Three different exposure times were used: 10-day tests (modified OECD 210 method), 4-day tests (modified ASTM test, 2004) and 21-day tests (nonstandard method) for *Oryzias latipes*, *Xenopus laevis* and *Physa acuta*, respectively. Capability of methods used to ecotoxicity assessment of reclaimed water, species sensitivity and correlations between embryotoxicity and analytical measures are discussed. This work has been funded by the Spanish Ministry of Economy and Competiveness through CTM2013-44986-R and CTM2014-52388-R projects.

TU015
Effects of the cytotoxic drug cyclophosphamide exposure in the polychaete Nereis diversicolor
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Anticancer drugs are known to have potent cytotoxic, genotoxic, mutagenic, carcinogenic, endocrine and teratogenic effects in several organisms, since they were designed to disrupt or prevent cellular proliferation, usually by interfering in DNA synthesis. After patient's treatment, anticancer drugs and their metabolites are eliminated and directed in urban effluents to wastewater treatments plants where removal is not fully accomplished. Therefore, those pharmaceuticals may reach the surface and coastal waters arising concern regarding their fate and long-term effects in aquatic organisms. The sediment compartment in aquatic environments represents an important reservoir of toxic and relevant contaminants, becoming also a source of contamination for water and biota. Standardized ecotoxicological assays used in risk assessment have not been applied to anticancer drugs and even less to sediment quality assessment. Hence, there is a lack of knowledge about the bioavailability and possible adverse effects over the benthic biota of coastal waters regarding chronic exposure to low levels of such cytotoxic drugs. Cyclophosphamide (CP) is a worldwide applied cytotoxic drug with one of the most potent alkylating activity in the DNA, able to interact to double strands of prokaryotes and eukaryotes. In this sense, the present study applies a multibiomarker approach including behavior impairments (burrowing kinetic), biochemical biomarkers and genotoxicity, assessed by the Comet assay, in the sediment-dwelling polychaete *Nereis diversicolor* exposed to the alkylating agent CP in water-sediment systems, over 14 days. A range of concentrations that cover environmental relevant levels and worst-case scenarios were used (10 ng.L^{-1}; 100 ng.L^{-1}; 500 ng.L^{-1}, 1000 ng.L^{-1}). Results showed impairments of burrowing at the lowest concentration of the drug (i.e. 10 ng.L^{-1}) and at 500 ng.L^{-1}. Antioxidant enzymes activity and DNA damage were altered at higher concentrations.

TU016
Genotoxicity assessment of environmental concentration of veterinary antibiotics in fish (Prochilodus lineatus) using the micronucleus test and others nuclear abnormalities
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Veterinary antibiotics are one of the most used pharmaceuticals class in Brazilian fish farming for the treatment of bacterial diseases, and because of their intensive use, contamination of the aquatic environment may occur with effects to the native biota which lives where the fish farming is located. The goal of this study was to investigate the genotoxic effects of environmental concentration of oxytetracycline **and florfenicol** on juveniles of native fish from Brazil (*Prochilodus lineatus*) through the occurrence of micronucleus (MN) and other nuclear abnormalities (NA) after 96 hours exposure. Environmental concentration of oxytetracycline (8000 ng L-1) **and florfenicol (425 ng L-1)** were determined in surface water of a fish farm located at Ilha Solteira Reservoir (Santa Fé do Sul, São Paulo, Brazil) through Online-Solid-Phase Extraction coupled to Liquid Chromatography-Tandem Mass Spectrometry (Online-SPE-LC/MS-MS) in a previous study of our laboratory and exposed along with its double (16000 ng L-1 **and 850 ng L-1**) and its half (4000 ng L-1 **and 212.5 ng L-1**) on juveniles of *P. lineatus* during 96 hours in a static system. Although MN **was** observed after exposure to the concentrations 4000 and 8000 ng L-1 **of oxytetracycline** no statistical differences were observed when compared to the negative control ($p < 0.05$). **No MN was observed after exposure to flofenicol concentrations.** Four kinds of others NA were observed after exposure to oxytetracycline, which are: notched nuclei, blebbed nuclei, lobed nuclei, and binuclei **and five kinds after exposure to flofenicol with were: notched, blebbed, lobed, broken eggs and binuclei, but in both cases no statistical differences were observed** as well as for the sum of abnormalities when compared with the negative control ($p < 0.05$). **Environmental concentrations of oxytetracycline and flofenicol are not genotoxic to *P. lineatus* according to the MN test.**

TU017

Effects of exposure to carbamazepine and ibuprofen on the mussel Mytilus trossulus from the Baltic Sea

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Pharmaceuticals in aquatic ecosystems are an emerging issue in environmental research and the lack of knowledge in literature on their possible effects on non-target organisms is evident. In order to examine biological effects induced by the common drugs carbamazepine and ibuprofen in the mussel *Mytilus trossulus* from the Baltic Sea, a semi-static laboratory exposure experiment was conducted. Consistent with available data on the residual concentrations of the compounds recorded in the Baltic Sea the mussels were exposed for 7 days to nominal concentrations of 40 and 160 ng/L of carbamazepine and 20 and 80 ng/L of ibuprofen, and to their respective low and high concentration mixtures. Measured concentrations of the test compounds in water samples taken prior to each renewal of the test media at 48 hrs intervals were ca. 70-85% of the nominal ones. As consolidated biomarkers of oxidative stress the enzymatic activities of catalase (CAT), glutathione reductase (GR) and superoxide dismutase (SOD) as well as lipid peroxidation (LPO) were measured from the digestive gland tissue. The phase II detoxifying enzyme glutathione transferase (GST) was determined from the same tissue while the activity of acetylcholinesterase (AChE), an indicator of neurotoxic effects, was measured in the gill tissue. Apparent effects on the oxidative defence system with marked changes in CAT, GR and SOD activities could be recorded after exposure to the higher concentrations of carbamazepine and the mixture whereas no significant effects could be shown for ibuprofen. At the lower concentration range the mixture seemed to be more harmful than the individually introduced compounds, suggesting a possible additive or synergistic effect. No significant changes in the AChE activity and LPO could be detected.

TU018

Detoxification of azoxystrobin via biotransformation in Gammarus pulex is influenced by co-occurring substances

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Xenobiotics, such as pesticides and pharmaceuticals are distributed ubiquitously in the aquatic environment and their co-occurrence is often reported. Combined effects may arise that can influence the detoxification of substances via biotransformation and as a result toxic parent substances accumulate in the organism. In the present study the strobilurin fungicide azoxystrobin (AZ) was chosen to study the influence of co-occurring substances on its biotransformation in the aquatic invertebrate *Gammarus pulex (G. pulex)*. AZ exhibits a generally high toxicity towards aquatic organisms by inhibiting mitochondrial respiration. The effect of an enzyme inhibitor and an inducer on the oxidative drug metabolism was investigated using two binary mixtures. The mixtures were composed of AZ and prochloraz as well as of AZ and carbamazepine. The imidazole fungicide prochloraz is known to be a strong inhibitor of cytochrome P450 (CYP450) whereas the antiepileptic carbamazepine acts as inducer of CYP450. For the identification of biotransformation products (BTPs) and to investigate temporal trends *G. pulex* were exposed solely to AZ (40 μg L^{-1} & 80 μg L^{-1}) and to the two mixtures (concentration of inducer and inhibitor: similar molar concentration as used for AZ). By using high-resolution liquid chromatography tandem mass spectrometry combined with suspect and non-target screening approaches 17 BTPs of AZ were identified. The major biotransformation pathway was an ester hydrolysis to its acid combined with a hydrogenation. Further reactions detected include hydroxylations and diverse conjugation reactions with glucose, sulfate or the combination of both as well as with glutathione resulting in cysteine products. Temporal trends of the internal concentrations of the substrate AZ and its BTPs were compared between the sole exposure to AZ and the mixtures. Carbamazepine did not show any inducing effect in *G. pulex* i.e. similar internal concentrations for AZ and its BTPs were observed in the substrate control and in the mixture. For the mixture, where prochloraz was used as inhibitor, a strong inhibitory effect was observed. Maximum internal AZ concentrations were about twice as high in the mixture compared to the substrate control as a result of biotransformation inhibition also reflected in the estimated biotransformation rate constants. As a consequence, mortality of *G. pulex* increased in the mixture compared to the single exposure to AZ, indicating raised toxicity.

TU019

Dietary exposure to PVP/PEI coated Ag nanoparticles in adult mussels causes abnormal embryo development in offspring

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In the last years, silver nanoparticles (Ag NPs) have gained high commercial interest basically due to their antimicrobial properties. Applications for Ag NPs are increasing and thus, concerns about their potential input into aquatic ecosystems and their environmental hazards are also growing. Toxicity of Ag NPs to different freshwater and seawater organisms is being widely studied; however, there is a gap of knowledge on the potential toxic effects of Ag NPs ingested through the food web, especially at environmentally relevant concentrations. Further, most investigations are focused on understanding the effects of Ag NPs on exposed organisms, but potential effects on offspring have not been explored yet. The aim of this work was to assess the effects of dietary exposure to PVP/PEI coated Ag NPs on mussels immune status, reproductive success and offspring embryo development. Mussels *Mytilus galloprovincialis* were fed daily with microalgae *Isochrysis galbana* previously exposed for 24 hours to two different doses of PVP/PEI coated 5 nm Ag NPs: an environmentally relevant dose of 1 μg/L Ag NPs and a high dose of 10 μg/L Ag NPs. After 21 days of exposure, Ag bioaccumulation was determined in different tissues and immunotoxicity was assessed in hemocytes. In addition, mussels were induced to spawn and sperm motility, spawning success, fertilization success and development of abnormal larvae were checked. Mussels fed with microalgae exposed to both doses of Ag NPs significantly accumulated Ag after 21 days, showing a successful transfer of incorporated Ag NPs from microalgae to mussels. Phagocytic activity of hemocytes was slightly reduced, contrary to the immunostimulatory effect reported earlier for Ag NPs and other NPs. No significant differences were observed for sperm motility, number of spawned eggs and fertilization success. However, a significantly higher percentage of abnormal embryos were observed after parental exposure to both doses, even for the dose close to environmentally relevant concentrations. Future studies are needed to address mechanisms and consequences of NP exposure for the early life stages of mussels and other organisms. Funded by: Spanish Ministry of Economy and Competitiveness (NanoSilverOmics MAT2012-39372), Basque Government (SAIOTEK S-PE13UN142 and Consolidated Research Group GIC IT810-13), UPV/EHU (UFI11/37 and PhD fellowship to N.D.) and French Ministry of Higher Education and Research (PhD fellowship to M.M.)

TU020

Including different biological and space complexity scales in hazard assessment : a case study with diclofenac sodium PNEC derivation

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Use of parent and metabolized drugs and their subsequent indirect emissions in aquatic ecosystems are a source of contamination of these and implies improvement of the scientific knowledge on their likely ecotoxicological impacts and of the tools to assess them. This is the goal of the DOREMIPHARM project, which aims at providing robust ecotoxicological data for drugs judged as of concern for their potential ecotoxicological risk as regards aquatic ecosystems. More widely, this research project also aims at developing robust hazard assessment tools for these pharmaceutical substances. The project involves different partners and their corresponding skills in order to implement a large number of tools allowing the testing of different conventional and non conventional endpoints (from a regulatory viewpoint). The project tasks allow data production for the implementation of an environmental risk assessment of the pharmaceuticals. The aim of this poster is to give an overview of the possible levels of assessment that may be taken on board for environmental hazard assessment of pharmaceuticals, focusing on the work done on diclofenac sodium. In fact, while traditional and regulatory Predicted No Effect Concentrations (PNECs) are usually based on the so-called “conventional” endpoints such as mortality and sublethal endpoints (e.g. growth, reproduction, development), it is here proposed to compare how the inclusion of less conventional endpoints may drive differently the hazard assessment. These endpoints depict different complexity scales as regards space (laboratory *versus* mesocosm data) and as regards biological organisation (community, population, individuals, cellular, biochemical). Applying this methodology, the so-called early-warning endpoints are compared to effects observed at community or populational level. Then, different PNECs are derived and presented : “traditional PNECs” covers conventional endpoints for at least three trophic levels (algae, crustaceans and fish), while “non conventional PNECs” may take account of the mesocosm study data obtained during the project but also of biomarkers response (e.g. biomarkers of effects such as immunotoxicological or respiratory burst effects).

TU021

How to investigate the impact of a multitude of micropollutants on the freshwater community? - Conceptual approach to address community level effects

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A multitude of micropollutants is regularly detected in freshwater ecosystems over the last years and there is an increasing concern that micropollutants – even if they were present in very low concentrations - may contribute to the losses of biodiversity observed in freshwater communities. In 2015 in North-Rhine

Westfalia (Germany) more than 90% of the freshwater ecosystems failed reaching the good ecological status, a target set by the EU's Water Framework Directive (WFD). Many micropollutants like pharmaceuticals, contrast agents, industrial chemicals, personal care products and some pesticides and biocides enter the aquatic environment by wastewater discharge, because these micropollutants are not or only partially eliminated by conventional wastewater treatment plants. Hence, wastewater is considered as one of the major sources of micropollutants in the aquatic environment. Bioassays conducted in the lab with representative species help to investigate the potential effects of complex mixtures of micropollutants on single species. In fact short-term effects of wastewater treatment effluents on different test organisms could be demonstrated by a set of bioassays in several research projects. Additionally, some bioassays conducted as by-pass test systems showed chronic effects of micropollutants on single species, too. Furthermore, monitoring results of freshwater communities in receiving water bodies before and after improvement of a wastewater treatment plant indicated that the quality of the introduced wastewater affects the macroinvertebrate communities. However, monitoring freshwater communities always includes many other influencing factors (e.g. temperature, velocity, habitat structure) which makes it impossible to analyse the effects of micropollutants alone. Therefore, it is still unclear how strong the multitude of micropollutants discharged by wastewater treatment plants will actually affect freshwater communities. We will present a conceptual approach of artificial stream mesocosms allowing to evaluate the impact of micropollutants alone without other influencing factors present in natural flowing water bodies. This approach allows us to investigate whether advanced wastewater treatment technologies like ozonation and activated carbon filtration will potentially improve the ecological status of macroinvertebrate communities.

TU022

Biological effects of anthropogenic pollutants present in pond receiving treated municipal sewage water

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In this study, we aim to assess biological effects of mixture of emerging pollutants, present in recipients of treated sewage water in common carp (*Cyprinus carpio*). Four samplings (after 0, 30, 90, 180 days) have been done in April-November, 2015. Cezarka pond, a biological pond which receives water from sewage treatment plant in Vodnany, Czech Republic (approx. 7000 inhabitants) has been studied. Anthropogenic pollutions such as persistent organic pollutants, pharmaceuticals and personal care products have been detected and identified in water and sediments. Fish were exposed in real condition at size of 17.05 ± 1.25 cm total length and 65.80 ± 15.12 g total weight. The effect of complex pollution on fish was identified. The oxidative stress and antioxidant enzymes activities were measured in liver, gill, muscle, and intestine fish tissues. At 180 days of experiment the weight of exposed fish was remarkably higher when compare to control group. Nutrient load in biological pond resulted in high abundance of plankton and macrophytes compared to control. It suggests that the growth was not affected by identified pollutants, while the difference may be due to amount of natural food available in Cezarka pond. However to understand the full picture of effects of anthropogenic pollution, it is necessary to consider other information regarding chemical, biochemical and reproductive endpoints. *Keywords:STP effluents,mixture effects, fish, biological pond* Acknowledgement: This work was financially supported by the Ministry of Education, Youth and Sports of the Czech Republic – projects "CENAKVA" (No. CZ.1.05/2.1.00/01.0024) and "CENAKVA II" (No. LO1205 under the NPU I program), by the Grant Agency of the University of South Bohemia in Ceske Budejovice (No. 012/2016/Z), by the Czech Science Foundation (No. 15-04258S).

TU023

Effects of environmental realistic concentrations of carbamazepine in a freshwater model ecosystem

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Pharmaceuticals are widely used in human and veterinary health care products. Their occurrence in the aquatic environment is nowadays a well established issue and has become a matter of both scientific and public concern. As these substances are biologically active and might be persistent, toxic effects on organisms are suspected at low concentrations and have been demonstrated mainly for steroidal hormones. Carbamazepine is a common drug used for the treatment of epilepsy, which is frequently found in European freshwater ecosystems. As chronic ecotoxicity data as well as information on the current distribution patterns in environmental compartments are scarce, an integrated hazard assessment strategy was proposed based on low tier (standardized tests, biomarkers studies) and high tier studies (lotic mesocosms). The results of the lotic mesocosm experiment are described in this communication. The study was carried out, in twelve 20 meter long channels, under continuous, environmentally realistic concentrations of carbamazepine (0.05, 0.5 and 5 μg L^{-1} in triplicats). The mesocosms were set up with artificial sediments, macrophytes, periphyton, benthic and pelagic invertebrates, decomposers and one fish species (*Gasterosteus aculeatus)*. After three months of ecosystem stabilization, treatment lasted 6 months. Periphyton biomass, macrophyte biovolume, zooplankton and invertebrate abundance and diversity, fish individual physiological responses along with population dynamics were the measured biological endpoints. Carbamazepine concentration in water was monitored each month by chemical analysis. In order to account for the observed effects, degradation products and major metabolites were also measured in the sediment, watercress and fish. Major effects on water quality parameters, macrophytes and fish were observed at the highest tested concentration. Moreover, a quite important physiological destabilization of fish was shown at 0.5 and 5 μg L^{-1}.All these impacts could be caused by both the parent substance and the degradation products and metabolites. Retroactive effects seem to drive and structure the responses of the other populations and communities. A conceptual model presenting the overall response at the highest tested concentration is proposed.

Ecotoxicology and risk assessment of nanomaterials - Grouping and read-across (P)

TU024

In silico models for nanoparticles: are we ready for regulatory application?

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The use of alternatives to animal testing such as *in silico* models based on Quantitative Structure-Activity Relationships, grouping and read across methods to reduce experimental costs and speed up risk assessment procedures, is a main innovation introduced officially by the REACH regulation in 2007. However, the need for identification of harmonized criteria for the evaluation of the reliability and applicability of QSAR models represented a real priority since the white paper on REACH, published in 2001. The approval and publication in 2004 of the OECD Principles for the regulatory use of QSAR, and the following OECD (2007) and ECHA guidelines (2008), represented milestones in the process of application of *in silico* strategies for the modelling and the prediction of properties and activities for traditional and industrial chemicals. These documents are based on experiences gained during decades of development, validation and application of QSARs to thousands of organic substances. Currently, the large development of nanotechnologies and the uncertainties related to possible effects and risks posed by these new materials to humans and the environment, have increased the interest of scientist and regulators in computational nanotoxicology, which is expected to provide key elements in the determination of missing data by *in silico* predictions. However, traditional schemes for models development and validation such as those described in the aforementioned OECD Principles, find difficult application to nanomaterials due to lacking input data, consequent limitations in the quality and dimension of the modelled datasets, and to a variety of factors, which strongly influence the behavior of nanoparticles. In this poster we analyze and present different issues related to the development and validation of computational QSAR-type models generated for nanoparticles. The analysis is performed on real datasets and models, following the OECD Principles for the regulatory use of QSARs and highlighting criticalities associated to the application of these five principles. The aim of this poster is to generate discussion and reflection on a sensitive topic such as the possible regulatory validity and use of predictions generated by *in silico* models developed for nanoparticles.

TU025

Are we ready for read-across approaches to support regulatory decision-making of nanomaterials?

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Regulatory jurisdictions continue to assess and manage potential risks associated with manufactured nanomaterials. In addition to assessing new nanomaterials, Canada has recently published an approach to assess and manage existing nanomaterials. To support domestic and international needs, approaches are needed to leverage existing information in risk assessments. These read-across frameworks (or approaches), which allow users to address gaps in data-poor substances with information from other nanomaterials, have become a priority for most jurisdictions. This need is driven by a combination of: short timelines to make regulatory decisions, conducting assessments which span different physical forms of nanomaterials, and the move towards reduced animal testing. However, many of the published read-across frameworks for nanomaterials are underpinned by principles of conventional chemicals. As such, these frameworks cannot be used for nanomaterials without first identifying the specific nano-properties of interest and second by conducting case studies to validate these approaches for nanomaterials. This presentation will provide an update on Canada's domestic regulatory program and activities under the Organization for Economic Cooperation and Development (OECD) Working Party on Manufactured Nanomaterials (WPMN) on the use of read-across in support of regulatory decision making. The intention of this presentation is to inform on the ongoing needs associated with read-across frameworks for nanomaterials while highlighting gaps which need to be addressed to improve the uptake of these approaches for regulatory decision-making in the future.

TU026

Reflect laboratory results the fate and effect of nanomaterials in the environment and represent appropriate data for grouping?

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The grouping of nanomaterials (NM) is based primarily on standardized, ecotoxicological laboratory experiments. However, whether data of laboratory experiments are comparable with data from field experiments has not been determined so far. The comparability of both is the basis for any grouping effort. The main goal of this study was to show if the data on fate and effects of a silver NM (AgNM: NM-300K) and a CeO_2 NM (CeO_2 NM: NM-212) from the OECD Sponsorship Programme in field lysimeters is comparable with laboratory results. Experiments with AgNM started in May 2014 and with CeO_2 NM in May 2015. Both will end in 2017. Eight field lysimeters (0.9 m x 0.9 m x 0.9 m) are used and various crops are planted. The NM were applied on the field lysimeters via sewage sludge (Ag, CeO_2) and simulated rain (CeO_2). For the application of NM via sewage sludge, sludge was removed from a municipal sewage treatment plant and mixed for 24 h with the NM. The sludge was dewatered and introduced into soil in accordance with the German Sewage Sludge Ordinance (5t per ha in 3 years, applied at once). For the application via simulated rain periodically CeO_2 NM dispersion was prepared over four weeks, which was applied in 2.5 liters of deionized water on the soil surface. The top 20 cm of soil were circulated to simulate the agricultural use of a plow. After the NM had been incorporated into the soil, wheat was sown at first and subsequently canola and barley. For the AgNM lysimeter, via sludge concentrations of 1.8 and 7.0 mg/kg dry soil were measured. On the CeO_2 lysimeters with the sludge application concentrations of 9 and 40 mg per kg soil were reached and applied as simulated rain 9 mg/kg soil were measured. Directly after the application and at the end of each growing period, soil samples were taken and the influence of the NM on the soil microflora was determined using the potential ammonium oxidation (ISO 15685:2012). In addition the influence on the growth of crops and the fate of the NM in soil and the plants was determined. The first results show that AgNM remain in the top 20 cm of the soil and can be found in low concentrations in the roots of wheat. The soil microflora is inhibited by the AgNM at the highest test concentration. There is a good comparability of the laboratory and the field experiments during the first 140 days. First results regarding the toxicity show, that the CeO_2 NM did not affect the soil microflora at test start (lab and field tests).

TU027

Grouping of nanomaterial and hazard assessment: requirements for additional criteria to assess the quality of studies

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In several occasions, scientists can predict the effects of nanomaterials that are in the same category. When possible, grouping is done to simplify analysis, decisions, and to help to select relevant assays. Fate categories could possibly be used to dictate the need for hazard tests or hazard profile to dictate environmental behavior. Nevertheless, to determine hazard in a regulatory framework, assays have to be conducted under defined guidelines and studies are assessed under a score system (i.e. the Klimisch score) to determine if information quoted are sufficient and incertitude acceptable. With this respect, as work is currently done to improve and adapt existing guidelines for nanomaterials, the need for additional quality criteria is emerging. In particular, nanomaterial identification is crucial in hazard assessment and grouping. A list of 10 parameters considered as necessary to identify nanomaterials. The aim of this study is a tentative to identify parameters needed for nanomaterials to validate a study, in order to improve the score system with adaptation for nanomaterials. Data are collected from literature for different nanomaterial tested (TiO2 and Ce for example) and analyzed. For each study, parameters assigned and results of test toxicity are reported. Parameters mainly linked to toxicity are tested using multivariate statistical analysis (PCA for example), which can help in identifying crucial parameters to estimate the quality of the study. Based on these results, a prioritization of additional parameter to be included when assessing the quality of a study using nanomaterials is proposed.

TU028

Ecotoxicological impacts of engineered nanoparticles in aquatic model systems

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The ecotoxicological effects of engineered nanoparticles (ENPs) are mostly unknown. However, their industrial use is continuously rising. Particularly, studies on prevalent and commercial relevant ENPs with innovative functionalized variations (e.g., coating or doping) are lacking. Therefore, the German Federal Ministry of Education and Research (BMBF) has launched the programme “NanoNature“ in 2008. This programme supports and promotes research projects aiming to understand the impacts of ENPs on the environment in general and on organisms in particular. As a part of NanoNature “DENANA” (“Design criteria for sustainable nanomaterials”) is a joint research project of 10 academic, industrial and regulatory partners that currently tries to conciliate the conflict of immediate marketing of innovative ENP-products and the precautionary principle regarding the potential environmental risks. Within this framework the Ecotoxicological Laboratory of the German Federal Environment Agency (UBA) is investigating the biological effects of differently functionalized ENPs (silicon dioxide, cerium dioxide and silver nanoparticles) on various aquatic organisms. Main objective is to provide a solid long-term data set on ENPs in aquatic ecosystems which can be used for hazard estimation and risk assessment in chemicals policy. Therefore, ecotoxicological tests with several species representing different trophic levels in aquatic ecosystems, ranging from green algae (*Desmodesmus subspicatus*) and higher macrophytes (*Lemna minor*) to invertebrates (*Daphnia magna*) and vertebrates (*Danio rerio*) started. Moreover, test designs for multigenerational studies with daphnids were developed to estimate the long-term impact of ENPs. Additionally, several analytical methods such as dynamic light scattering (DLS) to describe the particle size distribution of ENPs, the determination of zeta potentials and chemical analysis of ENPs in aqueous solutions began. Results will show if the currently used classification of the investigated ENPs as less toxic (e.g., SiO_2 nanoparticles) holds also for functionalized variations and will offer criteria for designing sustainable nanomaterials. Furthermore, experiments will add up of long-term data on the environmental exposure which provides a basis for regulatory concerns.

TU029

SOS-Nano: the Structure-Oxidative Stress relationship of metal oxide nanoparticles in the aquatic environment

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The Marie Sklodowska-Curie Action funded SOS-Nano project addresses one of the pressing issues of econanotoxicology: to investigate how the structural properties of nanoparticles (NPs) can be used to predict their potential toxicity in aquatic environments, the final sink of released NPs. The project is using the oyster (*Crassostrea gigas*) as a model organism to investigate the toxicity of a range of metaloxide (MOx) NPs exhibiting different physico-chemical characteristics. The experimental plan of SOS-Nano is designed to highlight the relationships between the physico-chemical proprieties of the model NPs (Mn_2O_3, CeO_2 and ZnO) and the toxic activity under the influence of natural water properties, and to rank their toxic potential through a multi-tier system combining genomics and functional measurements. The NOx NPs exposure settlements mimic different conditions of salinity and natural organic matter concentration, expected to influence NPs aggregation-stability-reactivity in marine environments; the characterization of the behaviour of model-NPs in the exposure settlements is complemented by the assessment of bandgap energies, dissolution and NP biological fate within target-organisms. The induction of oxidative stress in the oyster embryos is being determined in accordance with the *Hierarchical Oxidative Stress Paradigm*, which describes the progressive induction of cellular responses classified as antioxidant defence, pro-inflammatory effects and cytotoxicity. The toxic responses are being ranked through a multi-tier system combining genomics and functional measurements. The poster will present preliminary data on the physico-chemical proprieties of our chosen model NPs under different exposure scenarios and the extent to which they induce oxidative stress in the test organisms. This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No 655134.

TU030

Categorisation approaches and grouping for read-across of manufactured nanomaterials

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The Registration, Evaluation, Authorisation and Restriction of Chemicals regulation (REACH, EC No 1907/2006) is safeguarding the safety of manufactured nanomaterials (MNs) while at the same time encouraging the reduction of in vivo tests promoting in vitro and in silico approaches. Annex XI of the regulation provides for the application of grouping and read-across when testing is not considered scientifically necessary and categories can be properly defined in terms of similarity. The need to apply grouping approaches to MNs testing has been highlighted by several guidance documents from regulatory bodies given that assessing risks posed by all MNs on a case-by-case basis is challenging in terms of financial resources, ethical considerations and time demand. However, a reference guidance framework for MN read-across is not available yet. The ECHA Group Assessing Already Registered Nanomaterials (GAARN) has identified key issues and concepts to be explored in MNs grouping and read-across, including the need to consider properties beyond chemical composition (e.g. aspect ratio, particle size, shape, solubility, etc.), the identification of similarity rules in support of the application of REACH Annex XI to MNs, the relevance of toxicokinetic studies in grouping and read-across and in extrapolation from in vitro to in vivo tests. We have analysed the published literature on categorisation schemes and read-across for MNs. The proposed categorisation approaches cover a variety of assessment goals, including priority setting of MNs for further evaluation (including ranking based on level of concern) and guiding the choice of relevant endpoints and methods. In this paper we report what is available in the literature and identify research gaps, with a special attention to the provisions of REACH Annex XI in order to support further activities in this area.

TU031
Agglomeration behaviour of nanomaterials in environmentally relevant aquatic media - towards a nano-specific OECD test guideline
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Agglomeration behaviour is an important parameter affecting the environmental behaviour of nanomaterials. It depends on physicochemical characteristics of the dispersion media and agglomerating nanomaterial, its concentration and concentration of other substances in the dispersion. This study suggests experimental method (proposed as an OECD test guideline) and provides international regulatory guidance for testing nanomaterial agglomeration behaviour in environmentally relevant aquatic media. Proposed method determines nanomaterial agglomeration behaviour by analysing remaining particle concentration in the top 0.5 cm volume of the dispersion over a 6 hour time-period (0-6 hours). For a fully stable dispersion, this concentration did not change over time, while agglomeration and settling of nanomaterial gradually reduced the concentration of particles in this volume of the sample. TiO_2 (NM105) nanomaterial was chosen to study agglomeration behaviour in dispersions containing 0, 1, and 10 mM $Ca(NO_3)_2$ electrolyte and dissolved NOM (SRNOM, 30ppm). Agglomeration behaviour was studied in the presence and absence of CO_2 and at natural and established (5, 7, 8.5) pH. Resutls showed that dispersions of TiO_2 (NM105) were unstable proportionally to the amount of added electrolyte. Addition of NOM had a potential stabilizing effect on the system containing 1 mM $Ca(NO_3)_2$. Absence of CO_2 in the atmosphere above the analysed TiO_2 (NM105) dispersions did not improve or deteriorate the stability of electrolyte or electrolyte-DOM containing dispresions. Dispersions of TiO_2 (NM105) were unstable at pH values 5 and 7, closest to the PZC of TiO_2 (6.5). Dispersions at pH=8.5 revealed stability comapred to that of the systems analysed at natural pH. The decision tree, developed as a result of this study, provides the experimental routine to test the agglomeration behaviour of nanomaterials in aquatic media. Decision tree is designed to address the issue of nanomaterial agglomeration behaviour in such way that would require the minimal time- and cost- spending, through the combination of "dispersibility" and "dispersion stability" test approaches. Thus proposed experimental method allows not only observation of nanomaterial agglomeration behaviour, but also understanding of reasons and factors influencing this process.

TU032
Grouping of nanomaterials regarding aquatic ecotoxicity - hypotheses for selected NMs and experimental proof
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Due to the large variability of nanomaterials (NMs) the testing of every modification would require a tremendous amount of work and costs. Therefore, grouping of NMs is intended, limiting the number of comprehensively tested NM to a few considered as representative for a group. The NMs of each group have comparable characteristics with respect to the intended protection objective (e.g. ecotoxicity). Aims of this ongoing project are first the correlation of data of physico-chemical (PC) properties of selected NMs (Ag, TiO_2, ZnO, CeO_2) with ecotoxicological data and second the identification of suitable parameters as basis for grouping. Based on a literature review, for each of the selected NM-types parameters were identified which are expected to be relevant for ecotoxicological effects. For every NM-type 2 – 4 uncoated sub-NMs were selected (in total 12 NMs). The selected NMs were tested with algae (OECD 201), daphnids (OECD 202) and fish embryos (OECD 236), considering the modifications proposed for the testing of NMs. The NMs were characterized in Milli-Q-water and in the respective test media (OECD medium for algae, ADAM for daphnids, ISO water for fish embryos). Parameters such as surface size, crystalline structure, morphology, primary size, particle size distribution in dispersion, zeta-potential, dissolution, reactivity (CPH, ROS) were addressed. NM characteristics in water can differ significantly from those determined in test media. For example, for the spherical Ag NM (Æ 15 nm) the ion concentration after 72 h was in the range of 437 – 490 mg/L in water and the various test media. For a silver rod with a length of 3797 nm, the dissolution differed between 230 mg/L in water and 0.3 – 1.3 mg/L in the test media. The dissolution of a second silver rod (length: 2423 nm) in water and the test media differed by factor 10 (0.101 mg/L and 0.012 – 0.038 mg/L). Therefore, for a correlation of PC parameters with ecotox data only values determined in the test media should be used. For Ag, dissolution was expected to be the most relevant parameter. However, correlation between EC50 values and dissolution is weak. Differences in toxicity of the NMs were significantly lower than expected based on the dissolution. Daphnids proved to be the most sensitive test organisms although dissolution in the test medium ranked between the dissolution in the algae and fish medium. Additional PC-parameters which still have to be defined have to be considered for grouping.

TU033
A systematic investigation of the ENMs toxicity to freshwater organism: the search of the key physiochemical parameter
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Engineered nanomaterials (ENMs) are applied in more and more different products and areas. Therefore, concerns regarding the potential human and environmental impacts of ENMs have risen. However so far it is unclear, how the novel properties of ENM result in an increase of their toxicological potential compared to classical (microscale) substances. Then despite the increasing investigation to assess the potential risks of these emerging contaminants, a full characterization of the potential human and environmental impacts of these materials is still missing. As well, the physicochemical parameter which determines the toxic effect is still far to be clearly declared. A bibliographic survey concerning toxicity studies on the freshwater organisms representative for the trophic levels of Algae, Crustaceans, and Fish respectively, have been conducted for MWCNT, SWCNT, and Fullerene. This bibliographic survey resulted in ecotoxicity data-set on CNMs – organized according to: (1) Type of CNT; (2)Trophic level; (3) Physicochemical properties Length (nm), Primary Diameter (nm), surface area (m2/g), functionalization; (4) Use and type of dispersant; (5) System-dependent properties; (6) Ecotoxicity tests. All collected data has been analysed by applying the statistical tools as analysis of variance (ANOVA), correlation analysis and polynomial regression analysis. Thus, the correlation between physicochemical properties of CNMs and their toxic value as EC50s is here determined using *different statistical approaches*. Among the group of MWCNT, SWCNT, and fullerene respectively, different pyshicochemical properties have been found to have a significant effect on toxicity by the ANOVA analysis. The matrix of correlation coefficients approach shows, e.g. for SWCNT-crustaceans, that the exposure time and the length have a positive and strong correlation with the EC50 values whereas a negative and weak relationship with EC50 values. The lack of data in term of characterization of the ENM both in the media or in the commercial available form were the main limitations to the investigation. On our knowledge the approach here proposed is for its first time proposed and it might be useful as a first basic investigation for the identification of key parameters of relevance for the assessment of ENMs.

Ecotoxicology and risk assessment of nanomaterials - Interactions at nano-bio interface (P)

TU034
Potential on the application of multi-luminescent bacteria for ecotoxicological screening of nanomaterials
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A number of studies has investigated toxicological effects of nanomaterials with various methods and addressed the potential toxicity on different systems. Among the groups of nanomaterials, silver nanoparticles (AgNPs) are of concern for their potential biocidal effects of both ionized and particulated forms. In this study, four different surface-coated AgNPs, coating of citrate (Cit-), tannic acid (Tan-), poethylene glycol (PEG-), and branched polyethyleneimine (BPEI-), were selected to investigate toxicological effects. A luminous microbial array for toxicity risk assessment (LumiMARA) using multi-species of luminescent

bacteria was used to evaluate for those AgNPs. To date, various acute toxicity bioassays using bacteria for toxicity screening have widely been applied to understand the ecotoxicological impacts of pollutants on aquatic organisms due to their advantages such as simplicity, rapidity, and cost-efficiency. Using single-strain of bacteria, however, may have some limitations of the various sensitivity ranges compared to using multi-species of bacteria. To overcome this limitation, application of multi-species of luminescent bacteria as the potential ecotoxicological screening tool for surface-coated AgNPs was investigated. Different time-dependent inhibition rates for all tested AgNPs exposed to each luminescent bacteria were achieved and used for dose-response curves to calculate the values of the 50% effective concentration (EC_{50}). The values of EC_{50} for BPEI-AgNPs (1.57 to 5.19 mg/L) were lower than those for the other surface-coated AgNPs (i.e., Cit-AgNPs, Tan-AgNPs, and PEG-AgNPs). It appears that the toxicity of AgNPs could be activated by the interaction of positively charged AgNPs with the negatively charged bacterial cell wall. Effects of the sole coating materials were also investigated and achieved similar toxicological trends to surface-coated AgNPs, and consequently, coating materials may induce toxic effects of surface-coated AgNPs on the exposed lumenescent bacteria. The short exposure time of LumiMARA, 15 min, would be drawback for the applicability as the toxicological assessment of nanoparticles, which may have slow toxicity reaction. However, the advantages in simplicity, rapidity, cost-efficiency, and reproducibility of this bioassay may lead to have a potential applicability as an acute toxicity pre-screening tool for the environmental sample containing nanoparticles.

TU035

The effect of surface oxidation on the dispersion and biotoxicity of carbon nanotubes

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Carbon nanotubes (CNTs) are employed in a variety of applications due to their unique and extraordinary properties, causing increased discharge of CNTs into aquatic environments and thereby threatening aquatic organisms. However, the severe homoaggregation of carbon nanotubes (CNTs) due to their high hydrophobicity strongly limits their efficient utilization. Surface oxidation is considered to be an effective way to improve the dispersity of CNTs. In this study, we treated mutiwalled CNTs (MWCNTs) with concentrated H_2SO_4/HNO_3 to prepare surface oxidized MWCNTs (o-MWCNTs), investigated changes in surface properties of the MWCNTs after oxidation, and evaluated the effect of oxidation on the suspension stability and biotoxicity of the MWCNTs. The contents of oxygen and oxygen-containing groups including carboxyl, hydroxyl, and ester groups of the MWCNTs increased and the point of zero charge of the MWCNTs decreased with increasing oxidation time. The specific surface area of the MWCNTs increased as oxidation time increased due to the disclosed tube ends and the untied CNT bundles. The concentration of stabilized MWCNT suspension increased with ultrasonication time, and the o-MWCNTs had much higher stability than the pristine MWCNTs. Changes in surface property and colloid behavior of the MWCNTs by the oxidiation substantially altered their biotoxicity. The o-MWCNTs exhibited significantly higher toxicity toward the algae (*Chlorella Pyrenoidosa*) and bacteria (*Escherichia Coli*) than the pristine MWCNTs, and higher toxicity was observed for the o-MWCNTs with higher degree of oxidation. **Keywords:** Carbon nanotubes; oxidation; dispersion; biotoxicity

TU036

Effects of cationic polystyrene nanoparticles in the hemocytes of the marine bivalve Mytilus: role of soluble hemolymph factors

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Nanoparticles (NPs) released into the environment can undergo considerable transformations before reaching the target biological system: however, the evaluation of the biological effects of NPs requires additional understanding of how, once within the organism, NPs interact at the molecular level with cells in a physiological environment. In mammalian models, interactions of NPs with plasma proteins originate a coating known as protein *corona* (Monopoli et al., 2011, J Am Chem Soc 133:2525). The corona proteins control the specific cellular receptors for particle recognition, the internalization pathways, and the immune response. Formation of a NP corona has been recently reported also in terrestrial and freshwater invertebrates; however, no information is available in marine species. PS NPs can occur in the marine environment as degradation products of macro- and microplastics. Recent studies on the marine bivalve *Mytilus* showed that cationic PS NPs (PS-NH_2, 50 nm) affect different functional parameters of the immune cells, the hemocytes. In vitro exposure to PS-NH_2 in ASW medium decreased phagocytic activity and increased lysosomal enzyme release, extracellular oxyradical and nitric oxide production, with maximal effects at lower concentrations (1-5 μg/ml). Only at higher concentrations (50 μg/ml) lysosomal damage and induction of pre-apoptotic processes were also observed (Canesi et al., 2015, Mar. Environ. Res. 111:34). In this work, the effects of mussel hemolymph serum on the interactions between PS-NH_2 and hemocytes were evaluated. Cells were exposed for 5 - 60 min to PS-NH_2 suspensions in hemolymph serum (1, 5, 50 μg/ml). In the presence of serum, PS-NH_2 increased lysosomal damage and extracellular oxyradical production with respect to ASW medium. These effects were associated to an opposite trend in the activation state of p38 MAPK, a key component of immune signaling, in serum and ASW, respectively. Moreover, TEM observations indicate a general increase in cellular damage induced by opsonized PS-NH_2. The results show that soluble serum components can affect interactions of PS-NH_2 with *Mytilus* hemocytes, and suggest that a NP-protein corona might also be formed in biological fluids of mussels. The results underline the need of understanding how the formation of a NP protein corona may affect the biological outcome of in vivo NP exposure in marine invertebrates.

TU037

Exposure to sublethal concentrations of metal nanoparticles may lead to disturbed feeding behavior and elevated metal body burden of Daphnia magna

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In ecotoxicological studies of metal nanoparticles (MNPs), lethality is the most often addressed acute toxicity endpoint and aquatic crustaceans are often used as test species. Freshwater biota and *Daphnia magna* in particular, is one of the most vulnerable targets of MNPs mainly due to high sensitivity of *D. magna* to heavy metal ions, released from the MNPs, but also due to its physiology (e.g. feeding). Though significant for viability of the zooplankton population and the ecosystem health, sublethal effects of MNPs such as affected feeding are remarkably less studied. The aim of the current study was to evaluate whether the exposure to sublethal concentrations of MNPs affects *D. magna* feeding on microalga *Chlamydomonas reinhardtii* later on and whether there exists a risk for trophic transfer of MNPs (from *D. magna* to higher organisms). To evaluate the solubility-related effects of the studied MNPs, the respective soluble metal salts were studied in parallel. To obtain dose-response data, *D. magna* was exposed to Ag, CuO, Co_3O_4 and Mn_2O_3 NPs and the respective metal salts for 48h according to OECD202 guidelines. The tested concentration range for Ag NPs was 0,1-20 µg Ag/L and for other MNPs 0,01-100 mg metal/L. After the 48h chemical exposure, *D. magna* (from sublethal exposure concentrations) feeding on microalgae was assessed by algal autofluorescence in flow cytometry. In parallel, trophic transfer potential of metals upon exposure to sublethal concentrations of the studied chemicals was evaluated by quantifying the total body burden of metal in NP/salt-exposed daphnids i) immediately after exposure and ii) after post-exposure feeding using Total Reflection X-ray Fluorescence spectroscopy (TXRF). The 48h toxicity for *D. magna* decreased in the following order: Ag>>CuO>>Co_3O_4=Mn_2O_3 and $AgNO_3$>>$CuSO_4$>>$CoCl_2$•$6H_2O$>$MnCl_2$•$4H_2O$ for MNPs and the soluble salts, respectively. Co_3O_4 and Mn_2O_3 NPs were non-toxic up to 100 mg metal/L. Flow cytometry and TXRF proved suitable tools for studying the chosen endpoints. Results of the post-exposure feeding assessment showed decreased feeding of previously MNP-exposed daphnids compared to the metal salt exposed ones. TXRF analyses of MNP-exposed organisms showed that post-exposure feeding significantly decreased the total metal body burden nevertheless it remained elevated compared to that of unexposed control daphnids. Acknowledgements: This study was supported by Sciex NMS^{CH} project 13.143 (MH), by ETF9347 and by IUT23-5.

TU038

Effects of graphene oxide on aquatic macrophyte under the influence of humic substances

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The behavior of nanostructured graphene oxide (GO) in the environment is complex and must be completely understood to evaluate the risk involved. Studies show that humic acid (HA) can stabilize nanomaterials in water and change its behavior. The aquatic macrophyte duckweed (*Lemna minor*) is a good plant model for adverse effects evaluation of many test-materials due to its fast growth and small size. This study assessed the effect of GO, with and without HA, on the growth of *L. minor* on fronds number and biomass changes. The plants were exposed during 7 days to the nominal concentrations of GO equals to 0.0 (control), 0.1, 1.0, 10.0 and 100.0 mg L-1, in the absence or in the presence of 20 mg L-1 of HA. Tests were conducted in multiwell polystyreneplates where each well contained two fronds at the start of the experiment. The number of replicates for each test–concentration was 24 wells. The plants were exposed to a constant photoperiod (24:0, light:dark cycle) supplied by white fluorescent lamps (~700 lux) at the temperature of 20± 2 ºC. The fronds number was counted daily and the wet weight was assessed at the end of the experiment. The concentration that promoted 50% of inhibition in the growth rate in terms of fronds number ($EC50\text{-}7d_{growth}$) and the concentration that promoted 50% of wet weight reduction ($EC50\text{-}7d_{biomass}$) were calculated. The reduction of the growth rate by GO at the concentration of 100 mg L-1 was remarkable detected ($P < 0.001$) in the absence of the humic substance (HA). The HA seems to contribute with such reduction because this effect was also observed at the GO concentrations of 1.0, 10.0 and 100.0 mg L-1 in the presence of the humic substance ($p< 0.01$, $p< 0.001$ and $p< 0.001$, respectively). Although, the calculated $EC50\text{-}7d_{growth}$ for these exposures, with and without HA, was >100 mg L-1. This value attributes a practically non-toxicity for the test-material in terms of fronds production. On the other hand, the calculated $EC50\text{-}7d_{biomass}$ for the GO in the presence of HA or not was 2.8 and 2.4 mg L-1 respectively. These results indicate that the adverse effects of GO in *L. minor* are more pronounced on the biomass production since it was clearly observed that reductions in the fronds sizes were greater than in the fronds number. The $EC50\text{-}7d_{biomass}$ values assigns a moderate toxicity of GO to *L. minor* and these data may be useful for the establishment of maximum concentrations limits of this material in water bodies. **FAPESP proc 2014/01995-9, 2014/12891-0 \n**

TU039

Toxicity of engineered silver nanoparticles (NPs) is enhanced by co-exposure with clay particles in zebrafish larvae

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Naturally occurring particulates within the nano-size range are very abundant in surface waters and among these are mineral clay particles. Engineered nanoparticles (NPs) that are released into surface waters will undoubtedly interact with naturally occurring particulates; however, investigations of the effects of these interactions on NP toxicity have not been conducted. Clay minerals consist of silica tetrahedral (T) and alumina octahedral (O) layers in a lamellar structure that results in patch-wise surface heterogeneity with both positive and negative charges on their surfaces. The presence of both polarity charges on the surface of clay particulates suggests that interactions with silver (Ag) NPs with a negative zeta potential and dissolved free silver ions (Ag^+) will occur. We hypothesised that interactions between Ag-NPs and clays would alter aqueous phase Ag-NP toxicity in zebrafish *Danio rerio* larvae (ZFL). Parallel Ag-NP (0-3 mg/l) suspensions were prepared with and without 20 mg/l of Kaolinite clay suspension, and zebrafish larvae were exposed for 96 h (age 72-168 h post fertilization). Consecutive experiments were conducted with increasing concentrations of clay and humic acid (0-100 mg/l) and fixed concentrations of Ag-NPs (0.75, 1, 1.5 and 2 mg/l), and mortality of larvae was recorded. The clay exposed controls showed no toxicity from clay alone. Mortality of ZFL significantly increased when fish were exposed to both Ag-NPs and clay. The toxicity of 1 mg/l Ag-NPs increased with clay concentration by up to a maximum increase of 0.4 fold with 60 mg/l of clay, relative to 1mg/l of Ag-NP only. The 96-h Ag-NP exposure concentration predicted to kill 50% (LC_{50}) of ZFL with and without clay (20 mg/l) was 0.73±0.1 and 1.1±0.1 mg/L (±SEM) respectively, which is a 0.66 fold increase in toxicity within the clay and Ag-NP co-exposure. Humic acid significantly decreased toxicity of Ag-NPs (consistent with previous reports); however, the presence of humic acid did not eliminate the increased toxicity of Ag-NPs when co-exposed with clay. The observed increased toxicity of Ag-NPs in ZFL when co-exposed with clay is environmentally relevant and a novel result that suggests an alternative facet regarding the environmental effects of engineered nanoparticles.

TU040

Graphene oxide toxicological effects on daphnia and hydra

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The increased use of nanomaterials products requires robust strategies to identify risks when they are released into the environment. Graphene and its derivatives are promising candidates for biomedical applications and as potential advanced water purification agents. However, little is known about their ecotoxicological risks. Aquatic toxicity tests are performed with different aquatic organisms as daphnia and hydra in order to evaluate the risks resulting from the presence of graphene oxide (GO) in the environment. The following GO concentrations were tested: 0, 1, 10 and 100 mg.L^{-1}. Acute toxicity to D. magna was evaluated in a period of 48 hours when was recorded the number of moving subjects in order to determine the CE5024h and CE5048h (n = 24 / group). The percentage of daphnia presenting mobility after 48 hours of exposure was greater than 60% in all groups. There was a concentration effect of graphene oxide ($p = 0.02$) and mobility was 20% lower in group exposed to 100 mg.L^{-1} than the control group. To evaluate the occurrence of the effect of sublethal concentrations for 96 h, it was evaluate neonate *D. similis* growth rate through exposure to the same conditions used for acute toxicity testing (n = 10 / group). The bodies were photographed at the start of the test and every 24 hours. Also, there was studied the effect of the organisms mobility during 96 hours calculating the median survival time of the population, defined as the average time between the start of exposure and the immobility of organisms. There was a sharp decline when the organisms were subjected to 100 mg. L^{-1} GO. The *Hydra attenuata* test was performed for 96h. The organisms were observed every 24 hours for the presence of changes ranging from tentacles with bulbs (low toxicity) till shortening of the tentacles (severe toxicity), tulip aspect and disintegration (lethal). A mean score for each concentration was calculated. No mortality was observed in any concentration tested. However, it was observed some morphological alterations after 72h of 100.0 mg.L^{-1} exposure as clubbed and shortened tentacles and body slightly contracted. Exposures of the test organisms indicate that GO do not present a lethal risk or adversely affect them at concentrations lower to 10.0 mg. L^{-1}. Even though the results apparently demonstrate that GO currently do not pose a serious risk, it may exert some damage in aquatic organisms tested at higher doses. **FAPESP proc 2014/01995-9, 2014/12891-0**

TU041

Ecotoxicological effect of graphene on the benthic freshwater diatom Nitzschia palea

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Carbon-based nanoparticles such as graphene have many applications in different fields due to their outstanding electrical, optical and mechanical properties leading to their industrial production. Thus, few-layer graphene nanoparticles (FLG), composed of 1 to 4 planar carbon layers, are likely to be found in the environment and especially in rivers. In this study, the ecotoxicological effect of FLG on the freshwater benthic diatom *Nitzschia palea* was assessed. The experimental design used allowed to distinguish the shading effect from the total effect of exposure to FLG on *N. palea*,where diatoms are directly exposed to nanoparticles. A large range of concentrations was tested (0.1, 1, 10 and 50 mg.L^{-1} of FLG).To assess the effect of FLG on diatoms, several toxicity markers were evaluated such as growth rate, mortality and the photosynthetic activity. An inhibition of the growth rate of diatoms exposed to 50 mg.L^{-1} of FLG was observed 48 h after the beginning of the exposure, revealing a significant toxicity. In these conditions, the growth inhibition was associated with an increase in diatoms mortality. After 48 h of FLG exposure, a shading effect was observed on the cellular growth for culture exposed to the highest concentration of FLG. However, after 144 h of exposure, the growth rate was recovered, revealing a decrease in diatoms mortality, which can be explained by FLG stuck on the biofilm. *N.palea* naturally produces biofilm by excreting exo-polymeric substances (EPS) which can help diatoms to grow on the substrate. Indeed, EPS analysis by Alcian blue coloration and Scanning Electron Microscopy observation allowed us to conclude that EPS produced by diatoms strongly interact with FLG, sticking nanoparticles at the bottom of the well. These observations could explain the growth rate recovery at the end of the experiment in the total exposure conditions. In our experiments EPS secretion contributed to clarify the water column and reduced the physical interaction between FLG and diatoms to avoid the contamination. Overall, these results suggest that one potential toxicity process of graphene could be a combination of direct and shading effect leading to a strong interaction between biofilm and nanoparticles. Our results highlight the potential mechanisms of clarification of the water column by diatoms biofilms in natural ecosystems, by sticking FLG even at high concentration, which can correspond to an overflow accident condition.

TU042

Tissue specific responses of the Cape river crab, Potamanautes perlatus, to silver nanoparticles and temperature

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Silver nanoparticles (AgNPs) are incorporated into an increasing number of consumer products. However, the potential toxicity of AgNPs to aquatic organisms is largely unknown. The behaviour of AgNPs is influenced by several factors, such as the inherent properties of the NP (size, shape, surface area, surface charge, crystal structure, coating, and solubility/dissolution) and environmental properties of the dispersed media (temperature, pH, ionic strength, salinity, organic matter). In the aquatic environment, the collective effects of various stressors that act on similar pathways (such as toxicity, oxidative stress responses) confound the assessment of responses to NP. It is, therefore, important to study the effects of AgNPs in the context of other common environmental stressors (such as temperature). In this study, freshwater crabs were exposed to varying concentrations of AgNPs and varying temperatures for 7 days and the effects of a suite of biomarkers of oxidative stress were evaluated. Biomarkers of oxidative stress were investigated in the tissues (gills, hepatopancreas, haemolymph, haemocytes and muscle) in a freshwater crab species, the Cape river crab *Potamanautes perlatus*. A cytochrome P450 assay was used as an indicator of metabolism. Superoxide dismutase (SOD), catalase (CAT) and glutathione s-transferase (GST) assays were used as indicators of impacts on antioxidant capacity. The results indicated that the haemolymph are more susceptible to oxidative stress originated by AgNP and temperature stress, whereas the gills constitute the main storage organ for Ag. These tissue specific differences in toxicity to AgNPs reflect distinct nanoparticle effects. The results further concluded overall that AgNPs and elevated temperature in the environmentally relevant range increased the toxicity and cellular responses in the crab.

TU043
Bioaccumulation of Silver and Silver Nanoparticles in Earthworms
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Engineered silver nanoparticles (AgNP) are the most common form of nanomaterial found in commerce today. It is expected that these materials will end up in soils via the waste water stream through the application of biosolids to agricultural soils. Earthworms are an important part of the soil ecosystem and effects on their ability to reproduce or the possibility for bioaccumulation and subsequent trophic transfer is of concern. Three bioaccumulation tests were performed with *Eisenia andrei* in an agricultural sandy-loam soil: poly-disperse Ag nanomaterial comprised of 2 nm particles (0.3% PVP), silver nitrate ($AgNO_3$), and dispersed 40 nm AgNP (80% PVP) aged in biosolids prior to mixing with soil. For each test, *E. andrei* were exposed to the contaminated soil for an uptake period of 21 days and then transferred to a clean soil for an elimination period of 21 days. The results of the bioaccumulation indicate bioaccumulation factors (BAF) of AgNP > $AgNO_3$ > AgNP in biosolids (in order of significant bioaccumulation). In the cases whhere the soils were not amended with biosolids, the measured silver in earthworm tissue remained significantly greater than background after the elimiation period, whereas *E. andrei* in soils amended with AgNP contaminated biosolids eliminated the silver back to background levels.

TU044
Influence of surface characteristics on graphene oxide toxicity for zebrafish embryo
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Despite nanotoxicological studies have been intensified in recent years, gaps remain in the methods used to assess the nanotechnology risks. These gaps are due to the complex nanomaterials behavior in the environment, especially in the presence of organic matter and depending on the nanomaterial characteristics. Studies indicate that humic acid present in the aquatic environment can increase the stability of nanomaterial dispersions and may change its toxicity to aquatic organisms. The adsorption of oxidative debris (carboxylated carbonaceous fragments - CCFs) has also been discussed as an important factor influencing GO properties and behavior. The surface characteristics of GO can influence its biotechnological application as well as its toxicological effects. The aim of this study was to evaluate the influence of the presence of oxidative debris and humic acid in the toxicity of graphene oxide (GO) utilizing Fish Embryo Toxicity Test (FET test). GO (Sigma Aldrich) was refluxed with NaOH (0.01M, 1h, 90ºC) and HCl (0.1M, 1h, 90ºC) to produce GO without debris (GOwd). GO And GOwd were characterized through spectrophotometry, dynamic light scattering and atomic force microscopy. Zebrafish embryos (*Danio rerio*) were exposed during 96 h to 100 mg.L^{-1} GO or GOwd, in presence or absence of humic acid (HA, 20 mg.L^{-1}). A control group exposed to reconstituted water was also performed. At the end of the exposure period, the larvae were measured and frozen at -20ºC for subsequent evaluation of acetylcholinesterase activity (AchE). We also performed an *in vitro* test to evaluate direct effect of the nanomaterial in zebrafish AchE activity. Homogenates of zebrafish larvae were incubated with GO and GOwd at 30 mg/L (20 min, 30ºC) before AchE assay was performed. Both GO agglomerated and precipitated quickly in reconstituted water. The presence of HA in the medium stabilized the GO suspensions similarly to that occurred with GO in ultrapure water. There was no difference between groups related to the occurrence of embryo malformation or mortality. Larvae exposed to GO were shorter and showed lower AchE activity than control and group exposed to GOwd. The *in vitro* test showed that the nanomaterial did not inhibit AchE activity. The nanomaterial showed low toxicity to embryo, but the reduction in total length and AchE activity in the organisms can be due to indirect effects in zebrafish development. More experiments will be performed to understand those effects.
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TU045
Critical assessment of a 10 day sediment toxicity and bioaccumulation study of nano silver and silver nitrate using the polychaete Arenicola marina.
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There is an increasing use of Silver Nanoparticles (Ag-NPs) in various industry sectors, which are entering the marine environment via sewage treatment plants. Marine and estuarine sediments are thought to be a sink for the Ag-NPs as well as a food source for the polychaete worm, *Arenicola marina*. The two objectives of this study were to measure the bioaccumulation and toxicity of Ag-NPs to *Arenicola marina* and to assess the methodology used for sediment exposures for nanoparticles (NPs). Survival and cast production were assessed over the 10 day test duration. The toxicity and bioaccumulation of ionic silver was also assessed as a comparison. Chemical analysis of the sediment, overlying water and test organisms was conducted to detect silver concentrations using ICP-MS. The sediment silver concentrations ranged between 116 and 146% of nominal concentrations for the Ag-NPs tested. This shows that the particles remain in the sediment portion of the test system and are available to the test organism via ingestion of sediment. Silver was detected in the *A. marina* exposed to the higher concentrations tested, these treatments were also associated with low survival. Therefore there is uncertainty if the silver detected was due to bioaccumulation in the tissues or to sediment within the gut of the worms as depuration was not possible. The median lethal concentration (LC_{50}) is defined as the concentration resulting in 50% mortality of the *A. marina*. The Ag-NPs LC_{50} value for survival was 25.2 (22.5-28.3) mg/kg compared to 8.7 (5.2-14.5) mg/kg for ionic silver. The higher LC_{50} value for Ag-NPs suggests that they were less toxic than the ionic silver in this study. The Ag-NPs EC_{50} value for cast production was 13.9 (11.6-16.6) mg/kg compared to 10.4 (8.4-12.9) mg/kg for ionic silver. The second objective of this study was to assess the methodology and its suitability for nanoparticle toxicity assessment. The study used a direct spiking technique rather than solvent spiking to a dry sediment portion due to the nature of the test material. The spiking method was successful for this study, shown by the measured concentrations detected relative to the target values. However nanoparticles are produced in many different sizes, with and without coatings, in solid or liquid state and can agglomerate. All of these factors can pose problems when trying to spike sediments for toxicity testing therefore the specific approach may need to be adapted on a case by case basis.

TU046
Uptake of sediment-associated Ag nanoparticles in oligochaetes - a first step towards trophic transfer?
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Metal-containing engineered nanoparticles (Me-ENPs) are used in a wide range of products worldwide, such as inks, plastics, consumer products, lubricants, electronics and bioactive coatings. These particles will ultimately end up in the aquatic environment and have been shown to be taken up by a variety of species, from both water and sediment. Yet, research on potential trophic transfer of ENPs in aquatic ecosystems is limited. This study is the first in a series of experiments leading to the identification of trophic transfer of metal-based ENPs in freshwater environments. The main outcome of the study is the determination and quantification of ENP bioaccumulation in sediment-dwelling organisms, which will add to our understanding of this first step in the benthic food chain and thus of the potential trophic transfer of ENPs. Preliminary studies have shown that the oligochaete *Tubifex tubifex* accumulates Ag-ENPs from sediment after long-term exposures (28days). In this study, we investigate whether this bioaccumulation pattern also occurs after acute exposure (72-96h) to sediment spiked with different Ag-ENP concentrations. Ag-ions and a particulate reference are used to be able to pin-point potential nano-specific effects. The overall aim of the study is to determine the first step in food chain transfer of Ag-ENPs, using sediment as primary contaminant source. Considerations regarding acute vs. chronic exposure durations is made in relation to experimental design and bioaccumulation.
ICP-MS is used to analyze the degree of uptake in *T.tubifex*, estimated based on total metal uptake in organisms after exposure. The concentration range of Ag-ENPs is covering both low, environmentally realistic and higher, more response-inducing concentrations. Information on experimental setup, preliminary results and ideas for future research, including the next steps in the food-chain transfer investigation will be presented. We believe that by knowing if and to what degree aquatic organisms accumulate ENPs, a greater understanding of the actual risk and trophic transfer potential of these contaminants will be achieved.

TU047
Silver nanoparticles inhibit the embryonic development of Daphnia galeata
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Silver nanoparticles are widely used in various commercial and industrial applications; therefore, it is evident that they are already widely distributed in water environments. This study investigated the adverse effect of silver nanoparticles to the embryonic development of water flea. Test species is *Daphnia galeata*, which inhabits Holarctic lakes and rivers, and have sharp head, and are smaller in size than *Daphnia magna*.Embryos were extracted from *Daphnia galeata* adult female, and stage two embryos were selected, and exposed to a series of AgNPs concentrations for 72 hours. We measured a range of embryonic endpoints including antennae, eye, rostrum, heart, carapace, post abdominal claw, malpighian tube, sensory bristles, and tail spine. In addition, *Daphnia galeata* neonates were exposed to 50 nm of AgNPs for 48h hours. As a result, the EC50 value for neonate immobilization was estimated to be 38.42 ug/L. The 50 nm of AgNPs of 0.04 mg/L have serious inhibition of embryonic development. Further research is needed to investigate multiple species of water fleas which are important consumers in aquatic food chain. *Acknowledgements. This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (2013R1A1A2061386), and the Ministry of Science, ICT and Future Planning (2014R1A2A1A11050513).*

TU048

Investigating the Effects of Nano Copper Oxide on Plants, Collembola and Earthworms in Soil

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Copper nanomaterials have a wide range of uses in agriculture (biocides, wood preservatives), electronics (conducting material), household (biocide), and industrial (catalysts, conductive) applications. The variety of applications and usage can results in these nanomaterials ending up in the soil environment either directly (i.e., leaching of treated wood products or release from agricultural practices) or via the waste water stream through the application of biosolids. A suite of soil toxicity tests were completed to examine and compare the effects of nano copper oxide (nCuO) to ionic copper (Cu^{2+}, as $CuSO_4$) in a sandy-loam agricultural soil both with and without biosolid amendments. Plant tests examined effects on emergence and root and shoot growth (length and dry mass) of *Elymus lanceolatus* and *Trifolium pratense*. Invertebrate tests examined survival and reproduction in *Folsomia candida* and *Eisenia andrei*, as well as bioaccumulation for *E. andrei*. Tests were conducted at a range of test concentrations encompassing environmentally relevant concentrations at the low end as well as high concentrations in order to record effect levels. Test soils were measured for total Cu concentrations (HNO_3 digest/ICP-MS analysis) and Cu^{2+} activity (KNO_3 extraction/analysis by Cu^{2+} -selective electrode) to elucidate the source of observed effects as being a result of the nanomaterial or released Cu^{2+}.

TU049

Lifecycle and Impact of Sunscreens Using TiO2 Nanomaterials

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Among cosmetics and personal care products, sunscreen products are of emerging concern regarding both human and environmental health. The fate and impact of mineral nanoparticulate UV-blockers, such as TiO_2 nanomaterials, is under consideration from a regulatory perspective due to their potential impact. Once leaving the skin either through bathing or everyday usage and cleaning, the nanomaterials contained in the sunscreen can be released into rivers, lakes, sea shores, and/or sewage treatment plants. The nanomaterial behaviour, fate and impact in these different systems is largely determined by its surface properties, (e.g. the nanomaterial coating type) and lifetime. Here we present the first result of the Eco-SUN research program aimed at developing the eco-design of sunscreens through the minimization of risks associated with nanomaterials incorporated into the formulation. Different stages of the cream lifecycle are considered from its manufacture to its end of life, through its use by the consumer and its impact on the exposed environments. Reducing the potential release and / or toxicity of the nanomaterial from the cream is a decisive criterion for its eco-design. Different relevant TiO_2 UV-blockers have been selected to integrate a typical o/w formulation as case studies. The resulting sunscreen were characterised in terms of nanomaterial localisation, sun protection factor and photo-passivation. The risk for the consumer by dermal exposure was assessed using skin biopsies. Inflammation and skin penetration were evaluated. The risk for the aquatic environment directly expose was assessed both in terms of exposure and hazard. The release of nanomaterials from the sunscreen upon normal usage was studied in laboratory through simulated aging procedure. Two biological models, sea urchin and coral colonies, were selected as relevant endpoints to assess the marine ecotoxicity of the byproducts formed. Finally, the risk related to the end of life of the sunscreen through the removal with cleaning water followed by drainage to sewage treatment plants was evaluated by considering two opposite fate scenarios: (i) nanomaterial concentration in sewadge sludge later spread as fertilizer in agriculture, and (ii) nanomaterial suspension maintained in the treated water and released in river water. Thus, fate and impact in soil and river ecosystems were also studied.

TU050

Testing toxicity of graphene oxide in zebrafish embryos

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Due to their unique physiochemical properties, two dimensional graphene oxide (GO)-based materials have attracted considerable attention both in research community and industry. With the more application of this nanomaterial in the production of goods used in our daily life, it is increasingly released into the environment. However, the health risks associated with environmental exposure to GO are largely unknown, particularly with respect to embryogenesis. Zebrafish embryos is an alternative model for the evaluation of developmental toxicity of chemicals during early life stage with the characteristics of small-scale, high throughput and easy observations. In this study, 4 hours-postfertilization (hpf) zebrafish were treated with 8 concentrations of GO (water dispersion): 0.1, 0.3, 1, 3, 10, 30, 100 and 300 mg/L. A hole in the chorion was performed with a thin needle to prevent toxicity induction by hypoxia due to graphene adhesion to the chorion. Embryos were analyzed daily until 4 days post-fertilization (dpf) and not toxicity manifestations or lethality were observed at any of the concentrations tested. To guarantee the uptake of graphene, a second experiment in which grapehene was injected was carried out. Two concentrations were selected, 3 mg/L, the concentration from which aggregation of graphene was clearly detected in the first experiment and one lower concentration, 1 mg/L. Toxicity manifestations were not detected in this case either, what indicates that graphene is probably not toxic for zebrafish embryos, at least in the conditions used in this study.

TU051

Assessing the influence of cationic and hydrophobic modifications in the ecotoxicity of hydroxyethyl cellulose polymers.

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New highly charged polymers have been developed within the industry of personal care products, aiming at better performances for conditioning and deposit beneficial ingredients onto hair and skin. Among these, SoftCat^{TM} proved to be more efficient than conventional cationic polymers. They are polymers based on quaternary ammonium salts of hydroxyethyl cellulose and include several families of cationic products that differ in both viscosity and charge. SoftCAT SK comprise four high viscosity polymers that incorporate variations in charge level and hydrophobic modification while SoftCAT^{TM} SL constitute four high viscosity polymers that incorporate low levels of hydrophobic modifications. It has been shown that these variations in architecture correspond to different performances. In the present work is hypothesised that such differences in architecture are also associated with different ecotoxicological effects. If such association is detected the investment on the less toxic variation could be recommend to the industry. To test the above hypothesis, the following standard toxicity assays were carried out for each variation of SofCats^{TM}: bioluminescence inhibition with the bacteria *Vibrio fisheri*, the 72h-growth inhibition with the freshwater microalgae *Raphidocelis subcapitata,* fish embryo acute toxicitytest with the zebrafish(*Danio rerio*)and the acute immobilisation test with *Daphnia magna*. The results suggest that the variations of SoftCAT SL showed the highest toxicity in relation to SoftCAT SK in *Raphidocelis subcapitata, and Daphnia magna* species. Preliminary tests with zebrafish also suggest that the SoftCAT SL variation is the most toxic. However, further studies are still needed to fully understand the adverse ecological effects of these types of polymers and allow suggesting which comprise the more environmental friendly solution simultaneously retaining the desired functions. Keywords: Hydrophobic modification, cationic substitution, SoftCAT, environmental friendly.

TU052

Phytoavailability of TiO2 and Ag Nanoparticles

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The development of Nanotechnologies and the nanomaterial's integration in current products asks the question of the behavior and transfer of these new elements in the different compartments of the environment. This implies to carefully study the soil/plant tranfer of nanoparticles at environmentally relevant concentrations, and to model their phytoavailability as a function of soil, plant, and nanoparticle properties. The present work aims at estimating the impact of these various parameters (clay content, organic matter concentration, pH, coating, shape and size of nanoparticle) on phytoavailability at environmental estimated concentrations. Since hydroponic cultures do not allow studying the soil/nanoparticle or soil/plant interactions, we used the RHIZOtest (ISO 16198), a biotest that allows cultivating plants on soil separated by a polyamide mesh (pore size: 30 μm). Tall Fescue (Fetusca arundinacea) was in contact with several soils contaminated with TiO2 nanoparticles with different shape and size (shere 5nm, cube 35 nm, rod 250 nm), and Ag0 (PVP coated and SiO2 coated) compared to silver nitrate. The concentrations for TiO2 nanoparticles treatment were 1, 15, 150 mg/kg in soils. The concentrations for Ag nanoparticles were 1.5, 150 and 1500 μg/kg in soils. The results of the silver RHIZOtest highliht a soil effect with Ag nanoparticles in term of flux of Ag in the roots. The Ag fluxes with the two soils having the greatest cationic exchange capacity are smaller the two other soils. There is no significant difference between all nanoparticle treatments comparing to silver nitrate treatment. The dissolution of the nanoparticle will be checked by hyperspectral microscopy on roots. The results of titanium RHIZOtest show that there is no significant difference between controls and 1 and 15 mg/kg treatments.

The treatments at 150 mg/kg show that there is no effect of size and shape of TiO2 on titanium flux in plant. Furthermore, there is no significant difference of flux considering the type of soil.

TU053

Physiological and biochemical responses of polychaete Diopatra neapolitana to Graphene oxide

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In the recent years there is an increasing concern about the large number of emerging pollutants that have been released into the environment without yet being regulated, such as carbon based nanomaterials (CBN).Graphene Oxide (GO) is one of the most important CBN that has been extensively used but, such as for most of the CBN, limited literature is available regarding the impacts induced in aquatic organisms by this pollutant. The polychaete *Diopatra neapolitana* has frequently been used to evaluate the impact of environmental disturbances in estuarine systems due to its ecological and socio-economic importance but, to our knowledge, no information is available on *D. neapolitana* physiological and biochemical alterations due to CBN exposure. Thus, the present study aimed to assess the toxicity effects of different concentrations of GO (0.01; 0.1; 1.00 mg/L) in *D. neapolitana* physiological (regenerative capacity) and biochemical (oxidative stress related biomarkers) performance, after a 28 days exposure period. The results obtained revealed that the exposure to GO induced negative effects on the regenerative capacity of polychaete specimens, with individuals exposed to the highest GO concentartion (1.00 mg/L) presenting the lowest capacity to regenerate their body and lowest glycogen levels. Furthermore, with increasing of GO concentrations *D. neapolitana* increased the lipid peroxidation (LPO), Glutathione S-transferase activity (GSTs) content and decreased the ratio between reduced (GSH) and oxidized (GSSG) Glutathione. The activity of the antioxidant enzyme catalase (CAT) was not significantly affected by GO changes, although a slight increase was noticed at the highest GO concentration. The activity of the antioxidant enzyme superoxide dismutase (SOD) was significantly higher at the 2 lowest GO concentrations, comparing to the remaining conditions. Overall, the obtained results indicated that GO induced alterations in the regenerative ability of *D. neapolitana*. In addition, GO induced oxidative stress responses in this species, evidenced by changes in indicators of cellular damage and antioxidant defenses, specially noticed at the highest GO concentration. Thus, *D. neapolitana* demonstrated to be a good bioindicator to monitor emerging pollutants such as GO.

TU054

Co-exposure to titanium dioxide nanoparticles does not affect cadmium toxicity in radish seeds (Rafanus sativus)

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Nanoparticles (NPs) are currently present in a variety of industrial products and their use, disposal and fate (degradation and/or persistence) into the natural environment require urgent investigations. Recent developments on environmental fate models indicate that as *nanowaste* they could reach both terrestrial and aquatic ecosystems thus potentially affecting environmental and human health. Plants can be therefore exposed to NPs but controversial toxicity results in terms of fate and toxicity are currently available. Furthermore, there is a current lack of information on complex interactions/transformations of the NPs, which might occur in the natural environment as for instance interaction with existing toxic compounds. As a consequence the prediction and assessment of NPs behaviour, exposure and biological effects represent a current major challenge. In the present study, radish seeds have been exposed to titanium dioxide NPs (n-TiO_2)(1-1000 mg/L)(P25, Aeroxide) and cadmium chloride (1-250 mg/L) alone and in combination using a seed germination and seedling growth toxicity test in radish *Rafanus sativus* (OECD 208). Seed germination was not affected by n-TiO_2 alone but a significant increase in root elongation was observed but not dose-dependently. On the opposite, $CdCl_2$ cause a dose-dependent inhibition on root elongation and a complete abolishment of seeds germination at the highest concentration of 250 mg/L. Co-exposure clear showed no interaction of n-TiO_2 in Cd effects both at high (250 -1000 mg/L of $CdCl_2$ and n-TiO_2) and low doses (1-1 mg/L $CdCl_2$ and n-TiO_2). The presence of n-TiO_2 seems not affecting the bioavailability of $CdCl_2$ to seeds and roots so thus the toxic effects were still evident. Based on literature, n-TiO_2 (anatase) is known to adsorb Cd on the surface of the NP and thus potentially increase its bioavailability. According to our data, this was not observed since toxic effects exerted by $CdCl_2$ on *R. sativus* seeds were the same in presence or absence of n-TiO_2. DLS analysis showed that n-TiO_2 tested concentrations were quite dispersed in Milli-Q water reaching NPs size of ~180 nm (Z-average) and primary Z-potential (-31.7mV) and PdI (0.3). Seeds exposed to n-TiO_2 showed a clear uptake n-TiO_2 in particular on young leaf and inside the root. Our results underline that further studies are needed in order to address any potential interactions of NPs with toxic pollutants present in soils able to cause phytotoxicity in plants.

TU055

A network perspective on experimental data of organic matter and inorganic nanoparticles in aquatic media

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Understanding the influence of natural organic matter (NOM) on the stability of engineered nanoparticles (ENPs) is essential for understanding the behavior of ENPs in the natural environment, since NOM can stabilize ENP dispersions or enhance the aggregation of ENPs. The effects depend strongly on the physicochemical properties of the NOM and the surface characteristics of the ENPs. Therefore, to improve the mechanistic understanding of ENP-NOM interactions, experiments should study diverse combinations of NOM and ENPs, as pointed out by recent reviews. However, many of the numerous experiments carried out up until now repeat already well-studied NOM and ENPs combinations; rather few experiments investigate unique ENP-NOM combinations not studied before or after. This tends to lead to an overall low diversity of ENP-NOM combinations investigated, contrary to what is required. In this study we assess the overall diversity of this experimental field in order to identify knowledge gaps and possible research needs. Using the fact that several NOM and ENPs are common between the different experiments, we connected the materials that were investigated in 255 peer-reviewed papers that studied the influence of NOM on ENPs in aquatic media in a network. In this network, each node represents either an NOM or ENP of a given type, and the link connecting any two given nodes means that the two were used in the same experiment. In this network, many high-degree nodes are linked to low-degree ones, resulting in segregation of the data; in many cases, a given NOM or ENP is found to be tested with a set of ENPs or NOM types that is almost unique to it. The most central nodes are by far the Suwannee River Humic Acid for NOM and TiO2 and Fe2O3 for ENPs. This indicates that these constituents are investigated in combination with the largest number of corresponding constituents, in a large number of experiments. Temporal changes in the network's topology show a decrease in the global diversity over the last 25 years. In particular, we observe a decrease in the diversity of the NOM types employed in the experiments, which indicates that the focus is mainly on the ENPs studied. Overall, the trend observed implies that the empirical basis for a better understanding of NOM effects on ENPs remains rather limited, since insights from new studies are obtained for increasingly similar combinations of materials.

TU056

Biocompatible hydrogel nanocomposites for new generation drug design

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Medicine is the most exciting area for nanotechnology application even in such pivotal field of healthcare as cellular level fight against tumors. Despite its advantages, nanotechnology causes concerns, as nanoparticles can cause harm if applied in some particular fields. Dangers are connected to development of artificial materials with new properties. It is still unknown: how these new materials will "behave" in the human organism, as genetic after-effects might be displayed in the long-term perspective. Biocompatible hydrogel nanocomposites were synthesized by the method of frontal polymerization (FP) on the base of acrylamide and colloid solutions of locally available fossils: bentonite, diatomite, etc. Polydisperse bentonite powder was treated with solution of linear polyacrylamide as a surface active substance enveloping the nano-scale particles with their colloid state subsequent emission from polydisperse suspension. Such treatment allowed to synthesize polyacrylamide nanocomposite hydrogels with bentonite nanoparticles. The resulting nanocomposite hydrogel possessed appropriate adsorption properties and prolonged desorption of medicinal preparations according to preset programme. Adsorption and desorption optimal conditions were studied in such drugs as G?ngleron, Voltaren, Naltrexone at appropriate temperature and pH corresponding to acidity of human stomach and gastrointestinal tract. The kinetics of drugs controlled release was studied in conformity with established requirements. The most important advantage of synthesizing mentioned nanocomposites in FP mode is their absolute non-toxicity due to specific features of frontal method of synthesis. Unlike the globally used technology, due to specifics of FP, not only ecological compatibility is ensured at PAHs synthesis process, but toxic acrylamide is absolutely absent in obtained hydrogels. Due to their safety the possibility for wide use in various fields of human activity is created. The PAHs are cross-linked polymeric structures capable of swelling with absorption of a large amount of moisture and active substances without destroying the original shape. A specific advantage of PAHs is ability to release absorbed substances into the environment. It is noteworthy that this method has great advantages not only from the point of view of its high efficiency, ecological safety, possibility to control initial process parameters for the purpose to regulate properties of the obtained product.

TU057
Dispersion and aggregation of carbon nanotubes in aqueous solutions of anionic surfactants
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Dispersion and aggregation of CNTs in the aqueous environment are critical behaviors affecting their fate, bioavailability, and potential environmental and health risks. Bulk CNTs are generally aggregated and cannot be dispersed significantly in water or even in organic solvents. Surfactants are widely employed as dispersion detergents to prepare stably suspended CNTs for industrial applications. Therefore, for a better understanding of the mobility of CNTs in the environment, this work examined (i) whether bulk CNTs can be dispersed and stably suspended in the natural environment and (ii) whether surfactant-suspended CNTs can retain their stability in the natural environment. We observed that CNTs can be stably suspended in sodium dodecylbenzene sulfonate (SDBS) solution but not in water with assistant of sonication. Moreover, CNTs could not be dispersed and stably suspended in water and SDBS solution by shaking at 140 rpm, a mild agitation representative of the natural aqueous environment. Therefore, both SDBS and sonication play important roles in the dispersion of CNTs, with sonication breaking down large aggregates of SWCNTs, while SDBS adsorbed on the SWCNTs inhibits the coagulation and aggregation to maintain the stability of the suspension in water. Concentration of dispersed CNTs in the SDBS solution depended on the sonication energy, but not the sonication time or output power of the sonicator alone. The amount of dispersed CNTs was positively correlated with the concentrations of SDBS and CNTs, and the length of the CNTs. The optimal energy, i.e. the minimum energy supplied by sonication to achieve a saturated suspension of dispersed CNTs in the SDBS solution, was CNT diameter-dependent. Stably suspended CNTs in SDBS do not remain stable at the presence of cations (eg, Na+, K+, Ca2+ and Mg2+) after dilution. These observations suggest that CNTs will not travel long distances in significant concentrations in natural environment. Moreover, re-aggregation of suspended CNTs in the presence of cations was dependent on SDBS concentration but not CNT concentration.

TU058
Fate and uptake of nanopesticides in soil-earthworm systems
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Over the past few decades a significant body of work has been done to understand the ecological and health risks of synthetic pyrethroid pesticides (SPs). Recently the use of nano-encapsulated SPs has been proposed. As nanoparticles can behave very differently from dissolved chemicals, it is possible that the environmental fate, uptake and effects of these nano-formulated pesticides could be very different from the conventional SPs. This study therefore investigated the effects of nano-encapsulation on the fate and uptake of bifenthrin, a widely used third generation synthetic pyrethroid, in soil systems. Studies were performed, using OECD Guidelines, on five soil types with different properties (total organic carbon and texture) to determine dissipation half-lives in soil, soil-water partition coefficients and uptake and depuration in the earthworm *Eisenia fetida* using analytical grade bifenthrin, a conventional bifenthrin formulation (Capture LFR) and two nano-formulations. Persistence, sorption and uptake behaviour of all the study materials varied across soil types. Generally, the persistence, sorption and uptake of bifenthrin in the conventional formulation were similar to the behaviour of the non-formulated active ingredient. However, nanoencapsulation significantly affected the behaviour of the bifenthrin. Results for the two nanoformulations were similar to each other but these showed enhanced persistence, decreased sorption and increased rates of uptake and depuration in the earthworms compared to the analytical grade material and the conventional formulation. We therefore anticipate that the distribution and impacts of the nanoformulation in natural soil systems will be different from currently used formulations. The observed differences in persistence and sorption behaviour are possibly due to the polymer capsule 'protecting' the active ingredient from microbes and soil binding sites. Differences in uptake might be explained by differences in distribution of the bifenthrin within the organism (i.e. the nanoformulation is accumulating in the earthworm gut while bifenthrin in the conventional and non-formulated treatments is being internalised). *Keywords:* Synthetic pyrethroids; Nanoformulations; Bifenthrin; Earthworms

TU059
In vitro and in vivo effects of copper oxide nanoparticles and copper ions in Zebrafish (Danio rerio): Effects on Cells, Embryos and Fry
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The use of engineered metal nanoparticles (NPs) is continuously increasing and so is the scientific body of evidence regarding toxicity of these particles. However, further information on toxic effects of metal NPs is still needed to assess whether these particles are more or less toxic compared to their ionic counterparts. Here, we compare the toxicity of CuO NPs with ionic Cu to zebrafish hepatoma cells (ZFL), embryos and fry. In the *in vitro* tests no significant cytotoxicity (cell death, decreased metabolic or cell membrane integrity) was detected for Cu ions or CuO NPs, though both Cu forms caused significant effects on production of reactive oxygen species (ROS) compared to the control. However, results show that both Cu ions and CuO NPs are toxic to zebrafish embryos and fry. Embryo mortality increased in both Cu ions and CuO NP treatments. 24 h LC_{50}s was about10 μM (≈0.6 mg Cu L^{-1}) for both treatments, whereas exposure to Cu ions at 50 µM (≈3 mg Cu L^{-1}) caused significantly higher mortality than CuO NPs (≈100 and ≈70 % respectively). Only Cu ions showed effect on fry mortality (24 h LC_{50} about 30 µM, ≈2 mg Cu L^{-1} for Cu ions and >200 μM, 12.7 mg Cu L^{-1} for CuO NP). Both Cu treatments affected fry behaviour so that swimming activity increased during light cycles and decreased during dark cycles. In general, Cu ions had significant impact on behaviour at lower exposure concentrations than CuO NPs. In conclusion, there is a marked difference in toxicity among different levels of biological organization. Toxicity of Cu ions was higher than for CuO NPs for embryos and especially fry, whereas no significant toxicity was observed for either treatment to ZFL cells. This study shows that the release of metal NPs into the environment may have adverse effects on fish and possibly other aquatic organisms.

TU060
The interaction of silver nanoparticles with exopolymeric substances and its effect on algae
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Silver nanoparticles (AgNPs), as the most commercialized nanomaterial, are extensively used as bactericides or fungicides. As a consequence, AgNPs are increasingly released into the aquatic environment and potentially threaten plankton like algae. Exopolymeric substances (EPS) produced by algae may play an important role in the bio-nano interaction and nanotoxicity, which warrants to be investigated. We synthesized citrate- and PVP-stabilized AgNP suspensions (C-AgNPs and P-AgNPs, respectively), extracted EPS from a green alga (Chlorella.sp), investigated interactions between the AgNPs and the EPS, and specifically evaluated effects of the NP-EPS interaction on the algal accumulation and toxicity of the AgNPs. Results show that the algal EPS contained many active functional groups and strongly interacted with AgNPs as evidenced by the variation of UV and IR spectra, the increase in hydrodynamic sizes and zeta potentials, and the reduction in concentration of the released free Ag+ ions from the AgNPs in the presence of EPS. The EPS layer around algal cells slightly increased the algal adsorption of AgNPs but inhibited the cell internalization of AgNPs and alleviated the nanotoxicity. Compared with P-AgNPs, C-AgNPs had lower algal adsorption but higher Ag+ dissolution and cell internalization, and was therefore more toxic to the algae. This study highlights the importance of understanding the role of EPS in the bioaccumulation and toxicity of nanoparticles.

TU061
Impact of biosolid containing nanomaterials on a soil-plant-bacteria system
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Recent interest in the environmental fate and effects of manufactured CeO_2 nanomaterials (NMs) has stemmed from its expanded use for a variety of applications including fuel additives, catalytic converters, mechanical planarization media, coating and other uses.[1, 2] The majority of these NMs will end up in wastewater treatment plants (WWTP) where they will partition to sewage sludge during wastewater treatment, and ultimately re-enter the environment through the application of biosolids to agricultural soils.[3] Thus, soil may serve as a primary sink for NMs accumulation in the environment, in which NMs may enter food webs or cause direct toxicity to plants, microbial communities, or other soil organisms. Most of the available studies do not take into account environmental parameters such as the transformation of NMs in the environment or interactions between NMs and other contaminants. High concentration of trace elements (TEs) such as Cd, Pb, Zn, Ni are present in biosolids and can interact with NMs. With a large surface area for adsorption of TEs, NMs can facilitate the transport of TEs in soils[4] and modify their uptake by plant. This project aims to study the impact of Ce NMs on a soil-plant-microbe system using realistic exposure modes. Pristine CeO_2 NMs were first aged in a laboratory-scale activated sludge reactor during 5 weeks. The biosolid enriched NMs was then amended to a sandy loam soil (INRA's Couhins experimental farm, Bordeaux) at environmentally relevant

concentration: 1 mg Ce kg^{-1}. Canola plant (*Brassica napus*) was grown one month on this soil amended with the sludge without NMs or with biosolid enriched Ce NMs. Bulk Ce L_3-edge X-ray absorption spectroscopy (XAS) was used to study Ce speciation in the sludge before culture in order to evaluate the NPs transformation in the reactor. After the culture, elemental concentrations were measured in the plant parts by ICP-AES, and their distribution in roots by laser ablation ICP-MS. The first results indicate that the amendment of a biosolid polluted with CeO_2 NPs, performed at realistic concentration and after aging in a laboratory-cale reactor has an effect on the plant nutrients concentrations. Extensive analysis of elemental distribution in roots as well as effects on soil microbiota (microbial activities, bacterial community structure) are being performed in order to understand the impact of the enriched Ce biosolid and will be presented.

State of the science on poly- and perfluoroalkyl substances (PFASs) in the environment and humans (P)

TU062

A fast screening strategy for the analysis of poly- and perfluoroalkyl substances (PFASs) in solid matrices using laser diode thermal desorption coupled to high resolution mass spectrometry

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In recent years, concerns have arisen about the widespread environmental occurrence of poly- and perfluoroalkyl substances (PFASs) due to their potential for persistence, bioaccumulation and toxicity. Since the first specific analytical methods were published, significant advances have been made in the analysis of PFASs in complex matrices. However, conventional methods are relatively time-consuming to implement, requiring labor-intensive extraction and clean-up procedures, as well as a significant amount of sample material. The purpose of the present work was to develop a fast screening strategy for the analysis of legacy and newly-identified PFASs in solid matrices, using a reduced sample amount (< 200 mg) and a simple preparation procedure prior to instrumental analysis by laser diode thermal desorption atmospheric pressure chemical ionization (LDTD/APCI) coupled to high resolution mass spectrometry (Orbitrap-MS). The development of the analytical procedure was conducted with the help of experimental designs to identify optimum operating conditions, including the choice of a suitable extraction solvent, the amount of graphite used for sample clean-up, and LDTD instrumental settings. Following the addition of internal standards to the samples, an ultrasonic extraction step was performed using a small solvent amount (< 1 mL of methanol). For sample clean-up, graphite was then added (100 mg, batch) and the supernatant was recovered following centrifugation. 7 µL aliquots of sample were then analyzed by LDTD/APCI-Orbitrap-MS. The reduced sample preparation (no need of SPE manifold, no concentration step) and ultra-fast capability of the LDTD/APCI-Orbitrap-MS interface (acquisition time < 9 s per sample) imply that total analysis time (preparation + instrumental analysis) could be reduced to < 1 hour for 12 samples. The method was validated on fortified sediment and biota samples (i.e., assessment of detection limits, matrix effects, recovery, accuracy, and precision); when reference materials were available, method trueness was also evaluated. This allowed for the quantitative or semi-quantitative analysis of anionic, neutral and cationic PFASs in sediments and fish collected across Quebec at potentially-impacted sites (e.g., downstream from wastewater treatment plant effluents or aqueous film forming foam spills). Samples were also re-analyzed by LC/ESI-Orbitrap-MS, thus providing for a comparison with the newly-developed LDTD/APCI-Orbitrap-MS method.

TU063

Analysis of zwitterionic, cationic, and anionic fluoroalkylated surfactants in sediments by liquid chromatography polarity-switching electrospray ionization coupled to Orbitrap mass spectrometry

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The environmental prevalence of poly- and perfluoroalkyl substances (PFASs) in aquatic environments has been well documented. To date, perfluoroalkyl acids (PFAAs) such as perfluoroalkyl carboxylates (PFCAs) and sulfonates (PFSAs) have garnered the most attention of PFAS monitoring surveys. However, recent reports indicate that a considerable portion of the total organic fluorine in environmental samples may be attributed to PFAA precursors and other unknown PFASs. Here, we describe a screening strategy for the identification and quantification of a wide range of PFASs in sediments. A total of 28 model PFAS analytes were selected for optimization and validation purposes, including 21 legacy PFASs and 7 novel cationic and zwitterionic PFASs. Instrumental analysis was conducted by ultra-high performance liquid chromatography coupled to a Q-Exactive Orbitrap through a polarity-switching ionization source, allowing simultaneous acquisition of negative and positive mode PFAS within a single run. The extraction/purification step was optimized to maintain a common preparation procedure for all PFASs at once, and adequate whole method recoveries were obtained (60–110 % for 28/28 model PFASs). Method validation included assessment of blank contamination, linearity, detection limits, matrix effects, recovery, accuracy and precision. The newly-developed method was subsequently applied to a selection of riverine or lacustrine sediment samples collected at large spatial scale in mainland France covering the six French Water Basins. In addition to the 28 model PFASs used for optimization and validation purposes, these samples were screened for more than 60 infrequently reported anionic, zwitterionic or cationic PFASs. Perfluorooctane sulfonate (PFOS) generally prevailed over other PFAAs (concentration range of PFOS: 0.084–23 ng g^{-1} dry weight). Fluorotelomer sulfonamide amines (FTAs) and fluorotelomer sulfonamide betaines (FTABs) were also particularly prevalent in these samples. Hot spots of zwitterionic/cationic PFAS (estimated $\Sigma PFAS^{+}$ = 8.9–27 ng g^{-1} dry weight) were associated with low-flow watercourses in the close vicinity of airports, suggesting the existence of firefighting activities at these sites involving aqueous film forming foam (AFFF) formulations. In the long run, FTAs and FTABs could degrade to more environmentally persistent PFASs such as PFAAs.

TU064

Determination of perfluoroalkyl substances in marine water and seawater sandworm Perinereis nuntia by liquid chromatography-tandem mass spectrometry

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In this study, a high performance liquid chromatography-tandem mass spectrometry was used to determine 9 perfluoroalkyl substances in marine water and seawater sandworm *Perinereis nuntia*. The target PFASs were perfluoro-n-pentanoic acid (PFPeA), perfluoro-n-hexanoic acid (PFHxA), perfluoro-n-heptanoic acid (PFHpA), perfluoro-n-octanoic acid (PFOA), perfluoro-n-nonanoic acid (PFNA), perfluoro-n-decanoic acid (PFDA), potassium perfluoro-1-butanesulfonate (L-PFBS), sodium perfluoro-1-hexanesulfonate (L-PFHxS), and sodium perfluoro-1-octanesulfonate (L-PFOS). Marine water samples were filtered after collection and extracted and preconcentrated by solid phase extraction method. Sandworm *P. nuntia* was extracted using two methods – by manual extraction and by accelerated solvent extraction (ASE) and afterwards preconcentrated by the same SPE method that was used for marine water samples. Mass labelled PFASs (MPFAC-MXA) was used as the surrogate standard (SUR) added before the extraction of samples while M8PFOA and M8PFOS were used as the syringe spike internal standard to correct variations in both in extraction percentage and instrument response. Marine water samples extraction resulted to 57 to 115% method accuracy for all the target analytes. Manual extraction of sandworm was able to extract all PFASs (75 to 118%) while ASE extraction resulted in low method accuracy for short chain PFASs (i.e. PFPeA and PFHxA). Percent recoveries of SUR in marine water samples were between 52 to 72% (blank), 52 to 88% (1 ng), and 56 to 79% (10 ng). Manual extraction of sandworm resulted to 57 to 93% recovery of SUR compounds. ASE extraction gave higher recoveries of SUR compounds (62 to 92%). The developed methods can be applied to marine water and sandworm extraction to assure the quality of the data.

TU065

Development of Polar Organic Integrative Sampler for quantitative analysis of PFAS in surface waters

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Since the beggining of the 1990s, the development of passive samplers to analyze organic micropollutants in surface waters has been the subject of a large number of studies. They offer a complement to grab sampling, allowing for a better qualitative and quantititative analysis of a wide variety of contaminants. For instance, the Polar Organic Chemical Integrative Sampler (POCIS) has gained particular attention in recent years for the analysis of pesticides or pharmaceuticals. However, the application of this tool to poly- and perfluoroalkyl substances (PFAS) has been rarely reported, in spite of the ubiquitous character of these chemicals in aquatic environments. In this context, the present work consists in the development of a POCIS-like sampler allowing for the PFAS analysis in water, through a two-step approach. First of all, the selection of a suitable configuration was realised under controlled conditions for 12 PFAS (9 carboxylates and 3 sulfonates). Four configurations based on Strata-XAW or Oasis HLB sorbents enclosed between Nylon or PES membranes were compared. The second step consisted in the *in situ* calibration of selected configurations with a two-fold objective: to extend the list of targeted PFAS and to evaluate the environmental parameters that could influence the sampling rates Rs (necessary for quantitative analysis). The realibility of the tools for the quantitative analysis was evaluted in a last *in situ* experiment (7 days of exposure). The first experiment showed that only the Strata X-AW sorbent accumulated the 12 target compounds (in agreement with previsous report, PFBA was not accumulated by the HLB phase). The highest Rs were obtained with the Strata X-AW / PES configuration (Rs = 0.02-0.45 L/d). However, a lag-effect was observed with PES membranes because of the PFAS affinity for this material. Better linearity of PFAS accumulation (and, as a consequence, better quantitative analysis) was observed for long-chain carboxylates and sulfonates with the Strata X-AW / Nylon configuration (Rs = 0.08-0.29 L/d). The latter configuration was therefore elected, and subsequently calibrated in a periurban river near Bordeaux. 22 PFAS were dectected in grab and passive samplers. Sampling rates, similar to those obtained in a laboratory**,** ranged from 0.07 to 0.42 L/d and the linearity period was in the range 2.5-15 days. In situ assessment showed good agreement between concentrations obtained with grab and passive samplers.

TU066
Multianalyte profiling of per- and polyfluoroalkyl substances (PFASs) in liquid commercial products
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The specific chemical properties of per- and polyfluoroalkyl substances (PFASs) make them widespread for use in a number of industrial and commercial products. For instance, PFAS are known to occur in consumer products and are added to confer water and oil-repellency characteristics (e.g. waterproof, anti-stain) and to reduce surface tension in aqueous-film forming foams (AFFF). Some perfluoroalkyl acids (e.g., carboxylates and sulfonates), are known to cause significant human and negative environmental impact. Our current knowledge on the occurrence and content of specific PFAS in commercial products remains very scarce due to limited information available, thus impeding any precise assessment of human exposure and environmental release upon use. This study aimed at collecting 194 samples in Switzerland, and included a wide variety of liquid products likely to contain PFASs. Liquids tested included: impregnating agents, lubricants, cleansers, polishes, fire-fighting foams and other industrial products. By means of LC- and GC-MS/MS analytical techniques, a total of 24 different PFASs comprised of over 41 targeted compounds were detected and quantified in 55% of 194 samples. PFAS quantification and compound profiling was found to be consumer-product specific. PFASs were mainly found in AFFF (90% occurrence) and impregnating agents (60% occurrence) with more than 100 ppm of targeted PFASs in both cases. Mainly ionic (carboxylates, sulfonates) and neutral (fluorotelomer alcohols, acrylates) species were detected for AFFF and impregnating agents. Further investigation by Fast Atom Bombardment Mass Spectrometry (FAB-MS) on a set of AFFF samples allowed the characterization of 8 additional PFAS classes as major components in commercial AFFF formulations. Altogether, these results demonstrate the significant presence of numerous known and emerging PFASs in specific commercial products, which provide a source of further human exposure and environmental release.

TU067
Perfluorinated compounds (PFASs) in Northern Spain municipal solid waste landfill leachates
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Per- and polyfluorinated compounds (PFASs) are recognized as chemicals of environmental concern due to their ubiquitous presence in the environment. PFASs are highly persistent and some are bioaccumulative. They are used as surfactants, coatings and fire-fighting agents, as well as chemical components of an extensive array of consumer products that are ultimately disposed of in waste and wastewater systems. Given the potential for landfills to act as important environmental point sources of PFASs, the characterization of the PFASs present in landfill leachates is of paramount importance to provide details on loadings and sources to the environment. The few available data on PFASs occurrence in leachates correspond to landfills from North America, Europe and China. Recent surveys in China have revealed that leachates can contain levels of PFASs as high as 292000 ng/L. This work aims to study for the first time the occurrence of PFASs in leachate samples from municipal solid waste landfills in Northern Spain. The levels of eleven perfluoroalkylcarboxylic acids (PFCAs) and five perfluoroalkylsulfonates (PFSAs) have been assessed by high performance liquid chromatography and tandem mass spectrometry (HPLC-MS/MS) in four raw leachates and two treated leachates, ranging the total PFASs concentration from 562 ng/L to 2921 ng/L. PFASs concentrations were higher in treated leachates compared to their respective raw leachates, what suggests a possible degradation of PFASs precursors during treatment and the persistence of PFASs against biodegradation. Compounds like PFHpA, PFDA and L-PFBS were measaured in higher concentrations after application of the leachate treatments (biological and ultrafiltration). However, concentration of PFOA and L-PFOS diminished after the application of the leachate treatments. Overall, PFCAs accounted for the majority of PFASs, compared to PFSAs. Furthermore, short chain PFASs were detected with higher frequency, which could be caused by the production shift to products containing shorter-chain PFASs. PFOA was the pedominant compound in 3 of the samples (accounted 51-84%), L-PFBS was the pedominant PFAS in 2 samples and PFHxA was the most abundant in one sample (26%).

TU068
Comprehensive Monitoring of PFASs Precursors in Industrial and Municipal Wastewater Treatment Plants
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Perfluoroalkyl and polyfluoroalkyl substances (PFASs) are anthropogenic and broadly distributed via aqueous compartments around the world. Wastewater treatment plants (WWTPs) have been identified as a significant pathway for the introduction of PFASs to natural waters. Several studies, e.g. from 2006, 2010 and 2011 showed higher concentrations of PFASs analysed in the effluent of WWTP compared to the corresponding influent [1-3]. One reason for this might be the biotransformation of precursor substances, which were converted into known and analysed PFASs. Precursor substances can be fluorotelomer compounds, such as fluorotelomer alcohols (FTOHs), which have been shown to degrade to perfluoroalkyl carboxylic acids (PFCAs). Therefore, a comprehensive study was carried out in the framework of a project funded by the German Environmental Agency, sampling six municipal and industrial wastewater treatment plants located in Europe. A total of 65 PFASs were monitored using HPLC-ESI-MS/MS and GC-EI-MS methods. For wastewater treatment plants, eight influent samples and four effluent samples were taken over a period of four weeks. Additionally, eight corresponding air samples above the influent were taken in order to verify the presence of volatile PFASs as well as four grab sludge samples to account for adsorbable PFASs. Various findings of both, precursors, biotransformation intermediates and stable PFASs will be presented and discussed.

TU069
Black guillemot sheds light on local pollution in the Arctic: Levels, profiles and effects of PFAS
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The auk Black guillemot (Cepphus grylle) is a year-round resident species at Svalbard. Since it does not migrate south during the winter season, we can expect that all contaminant uptake comes from the local diet. Hence, the Black guillemot may help shed light on local pollution. Over the past 18 years, levels of legacy contaminants have decreased in another auk, Brünnich guillemot (Uria lomvia), which is monitored at Svalbard and Jan Mayen (MOSJ). However, the Arctic is experiencing an increasing input of emerging contaminants such as perfluorinated substances (PFAS); The Brünnich guillemots restrict themselves to the northern hemisphere but migrate during winter, spreading across the North Atlantic; Iceland, Faroe Island, Greenland and Canada where they might be exposed to different levels and cocktails of contaminants. As a result, we know little of the levels and profiles of legacy and emerging contaminants in species that spend their whole year feeding in the same area, such as the Black guillemot. The Black guillemot is a diving seabird foraging on small fish and crustaceans, closely related to the Brünnich guillemot, and hence resembling each other both ecologically and physiologically. Therefore, by comparing our results with available MOSJ data for Brünnich guillemot, we expect similar levels, but different profiles due to differences in foraging areas and contaminant uptake throughout the year. In addition, there is little knowledge about how the seabirds are affected by the contaminants regarding both concentration levels and chemical mixtures. This knowledge gap will be investigated in this study, through the measurement of DNA damage using the comet assay in Arctic seabirds in relation to contaminant body burdens. Contaminants may influence several steps in the antioxidative defense system and thus lead to increased oxidative stress and possible damage to DNA. Measured DNA damage occurring in Black guillemots will be used to establish a baseline, linking DNA damage to PFAS levels in the same individuals. Blood was collected from Black guillemots (N=15) in Kongsfjorden, Svalbard, summer 2015, and were analysed for PFAS as well as legacy contaminants, diet signatures and DNA damage, using the comet assay. Our questions were: 1) How does PFAS levels and profiles in a resident species differ from migrating species at comparable trophic levels? 2) How are contaminants levels and profiles linked to the amount of DNA damage observed in the Black guillemot?

TU070
Perfluorinated compounds in water, sediment and wild bird eggs from the Orange-Senque River basin, South Africa
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Currently, data concerning Perfluorinated Compounds (PFCs) is limited for the

South African environment. This study focussed on the analysis of selected PFCs from multiple environmental matrices in the Orange-Senqu River basin, one of the largest river systems in South Africa. Extraction and clean-up methods employed, varied for each matrix. Abiotic samples were extracted using sodium hydroxide followed by weak anion exchange solid phase extraction (SPE). Biotic samples were extracted using methanol in combination with dispersive activated carbon SPE clean-up. Thereafter, the extracts were analysed using liquid chromatography tandem mass spectrometry (LC-MS/MS). The mean recoveries for PFCs varied between 18 – 105% for water; 33 – 122% for sediment, soil and tailings dam waste; and between 45 – 137% from bird eggs. Perfluoroheptanoic acid (PFHpA) and perfluorohexanesulfonic acid (PFHxS) were not detected. Only 8% of water samples analysed had quantifiable PFCs, while soil, sediment, and tailing matrixes also showed a low frequency of occurrence with only 24% of samples containing PFCs. Measurable concentrations within the aquatic environment were expeted where the wild bird eggs were collected. Ninety percent of the bird eggs analysed had quantifiable concentrations of PFCs. PFOS had the highest prevalence, followed by perfluorodecanoic acid (PFDA) and perfluorononanoic acid (PFNA). The concentrations of PFOS in eggs of the same species from the same site were quite variable, probably due to their wide feeding ranges. This variability was further investigated by ensuring the validity of analytical identification, as well as looking at unique environmental exposure routes. PFOS identification was confirmed by retention time, mass transition, and isotope modelling against an authentic PFOS standard. Acknowledgements: Financial assistance was provided by the Department of Trade and Industry, the United Nations Office for Project Services and the National Research Foundation, South Africa.

TU071
PERFLUOROALKYL SUBSTANCES (PFASs) IN REMOTE HIGH ALTITUDE AREAS AROUND THE GLOBE
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Perfluorinated alkyl substances (PFASs) are compounds of environmental concern. Of the numerous PFAS investigated in environmental samples, perfluorooctane sulfonate (PFOS) is listed as persistent organic pollutant and recently the German Environment Agency submitted a proposal at ECHA to restrict perfluorooctanoate (PFOA). In the past, several perfluorinated acids have been detected in snow or firn of high altitude regions in Central Europe. In the present study, we investigated the presence of these compounds in snow and water samples at altitudes up to 5000 m a.s.l. at 8 remote sites. These were located in South America, Asia, Northern and Central Europe. Snow and lake water samples were taken in duplicate and one field blank was taken per site. Whenever the amount of melted snow was too small, samples were combined. PFASs were detected at all sites in water and/or snow samples. Highest PFAS snow concentrations were observed in Slovakia (High Tatras) and Italy (Monti Sibillini) with perfluorononate (PFNA) being the compound which contributed the most. Lowest concentrations were observed in China. Highest PFAS concentrations in lake water samples were observed in Switzerland (Macun Lakes) followed by Russia (Altai) and Slovakia (High Tatras) usually with PFOA contributing the most. The occurrence of PFASs at high altitude lake and snow samples demonstrates the ubiquitous distribution of these compounds and indicate their atmospheric transport to remote regions, probably by degradation of atmospherically transported volatile precursors.

TU072
Accumulation and temporal trends of PFCs in finless porpoises (Neophocaena asiaeorientalis) collected from Korean coastal waters between 2003 and 2010
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Perfluorinated compounds (PFCs) are ubiquitous contaminants in marine environmental compartments such as seawater, sediment, and biota. However, limited information is currently available on temporal trends of perfluorinated compounds (PFCs) in marine mammals from Korean coastal waters. In our study, accumulation and temporal trend of perfluorinated compounds (PFCs) were examined in liver samples of finless porpoise (*Neophocaena asiaeorientalis*) collected from Korean coastal waters between 2003 and 2010. Total concentration of PFCs (∑PFC; sum of 3 sulfonates, and 12 carboxylates) ranged from 123 to 1322 ng/g wet weight (ww) (median: 447 ng/g ww) for 2003 (n=27), and 245 to 3690 ng/g ww (median: 840 ng/g ww) for 2010 (n=77). In both years, PFUnDA (C_{11}) showed the highest contribution, followed by PFOS (C_8) contributed collectively over 70% of the ∑PFC concentrations. Other longer-chain carboxylates such as PFDoDA (C_{12}), and PFTrDA (C_{13}) also had relatively higher contributions than other PFCs. To investigate age-, and sex-dependent accumulation pattern, specimens in 2003, and 2010 were divided into 4 groups (immature male/female, and mature male/female). The median concentrations of PFCs were higher in immature female followed by immature male, mature male, and mature female in both years. In 2010 male specimens, as body length increases, the concentration of PFCs showed decreasing trend ($p < 0.05$), suggesting biodilution effect of PFCs. The ∑PFC concentrations of mature females in both years were lower than immature male/female, suggesting the excrection of PFCs via placental transfer and lactation. Compared to 2003, the ∑PFCs concentrations were significantly increased in 2010 ($p < 0.0001$). Among major compounds (which contributes >5% to ∑PFC concentration), PFOS (182%) showed the highest increasing rates than other compounds (PFUnDA: 73%; PFDoDA: 82%; PFTrDA: 40%). Our result suggests that a certain time would be needed for phase-out of PFCs in marine mammals due to the on-going use of products containing PFCs.

TU073
Particle-Induced Gamma-ray Emission (PIGE) as a Novel Screening Method for PFASs in Groundwater
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Because of the environmental persistence of per- and polyfluoroalkyl substances (PFASs), their ability to bioaccumulate, and their suspected human toxicity, new methods to identify these chemicals at trace levels in groundwater are needed. Particle induced gamma-ray emission (PIGE) spectroscopy is an established ion beam analysis technique that has been used to quantitatively measure light elements, including fluorine, in diverse target materials. An *ex-vacuo* PIGE method has been developed and is shown to be an effective and sensitive tool to determine the total organofluorine content in groundwater. Solutions of various chainlength perfluorinated acids and sulfonates were extracted onto the surface of a weak anion exchange solid-phase extraction (SPE) cartridge. This pre-concentration method was sufficient to use realistic volumes (40 - 80 mL) of distilled water to measure total fluorine. The total fluorine measured on these cartridges by PIGE correlates well with the initial PFAS concentrations for a broad range of anionic PFASs. Subsequent experiments with actual contaminated groundwater from two US sites further demonstrate PIGE to be an inexpensive, rapid, and non-destructive method for total fluorine analysis. Spike and recovery tests have been performed as well as a post-extraction method to remove fluoride ions from the cartridges. Thie novel spectroscopic method for detection of PFAS can be adapted to detect environmentally-relevant PFAS concentrations in groundwater and may be very suitable as a pre-screening method to determine the presence or absence of PFASs in groundwater samples.

TU074
What do we know about indirect exposure to PFOS? In vitro metabolism of MeFOSE and MeFOSA
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Perfluorooctane sulfonate (PFOS) is a widely known Persistent Organic Pollutant (POP) with an extensive historical use, until its restriction in the early 2000s. Due to its proven toxicity and health concerns it was incorporated in the Stockholm Convention on POPs in 2009. After the phase out by the principal manufacturer (3M) in 2000-2002, trends in direct exposure to PFOS have been reported to decline. However, the indirect exposure through PFOS-precursors that are metabolized to PFOS has been suggested in recent literature. This PFOS indirect exposure could be an increasingly important contribution to the levels of PFOS in biological samples: estimates derived from modelling studies range current exposure from these PFOS-precursor compounds between 10% and 40% of total PFOS body burdens. Such PFOS-precursor compounds include perfluorooctane sulfonamides (FOSAs) and sulfonamidoethanols (FOSEs), which historically were synthesized to produce polymeric materials and phosphate esters, for their later use on surface coatings for textiles and paper products. However, there are still several uncertainties associated with these estimates and metabolism of not all PFOS precursors have been investigated so far. So, further *in silico*, *in vitro* and *in vivo* studies of these compounds are strongly required, as they would render the margin of safety between the current exposure limits and estimates of external exposure to PFOS alone. In the current work, the *in silico* and *in vitro* metabolism with human liver microsomes (HLM) and cytosol (HLCyt) of two of these “PFOS-precursors” ((N-methylperfluoro-1-octanesulfonamido)-ethanol (MeFOSE) and N-methylperfluoro-1-octanesulfonamide (MeFOSA)) were investigated. Results show at least 4 expected metabolites for MeFOSE and 5 for MeFOSA (eg. PFOS, FOSA, hydroxylated metabolites, break-down products and conjugations).

TU075
The remediation of PFAS contaminated airport soils
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AFFF has been used historically at Norwegian airports and as such has resulted in contamination of ground water and soils with a multitude of per- and polyfluoroalkyl substances. The behaviour of PFASs in the unsaturated soil zone and the way in which this affects contamination of underlying groundwater is relatively unknown. In order to suggest suitable methods of remediation that comply with environmental legislations it is important to obtain an understanding of this behaviour. In Norway very low target values for the concentration of PFOS that are permitted in water and soil drive remediation efforts. Cleaning up to such low concentrations is technologically, logistically and chemically challenging. Here we present a feasibility study for the remediation of a soil from a Norwegian airport and contaminated with PFOS. Soil samples were taken from a currently used firefighting training facility and the concentration of a variety of PFASs was quantified. Concentrations of PFOS ranged from 6.4 ∃g/kg up to 2600 □g/kg and all of the soil samples were sandy in nature. Lab experiments were carried out to investigate leachability using a shaking test. The standard shaking test involves mixing the soil with water at a L:S ratio of 10 and revealed that the leachability of PFOS was up to 100 % of the total concentration. For both the total concentrations of PFOS and the leaching there were no clear trends with soil properties (K_D, organic matter content, Ca content, Cl content, pH, Mn content, Fe content or SO4 content). Soil-water partitioning coefficients were calculated and were low for these samples (2 to 50 L/kg). Remediation trials were tested in the lab for a possible field clean-up effort and they consisted of soil washing to remove a great deal of PFOS and stabilisation to immobilize the remaining PFOS. Activated carbon, a clay and an organic matter rich compost soil were tested as the sorbents for stabilisation. Soil washing involves the addition of a vast quantity of water and a separation of different soil fractions. The presentation will show the results of these trials which will be instrumental in order to suggest the most feasible site remediation options in collaboration with the Norwegian Airport Authority and the Norwegian Environment Agency.

TU076
Performance and hazard assessment of fluorinated and non-fluorinated state-of-the-art DWR-polymers
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Durable water repellent (DWR) impregnation is applied in textile finishing to impart water and, depending on impregnation chemicals, oil and stain resistance to textiles. Following the phase-out of the most effective and predominating DWR-technology based on long-chain per- and polyfluoroalkyl substances (PFASs), the textile industry had to find suitable alternatives. This phase-out has resulted in a market where both fluorinated and non-fluorinated DWRs are available, dividable into three broad groups: short chain PFAS-based, silicone-based and hydrocarbon-based polymers. During our research in the SUPFES (Substitution of prioritised poly- and perfluorinated chemicals to eliminate diffuse sources) project, the alternative DWRs were assessed with regards to: (i) their structural properties and connected performance, (ii) loss and degradation processes resulting in diffuse environmental emissions, and (iii) hazard profile for selected emitted substances. We worked with DWR-chemistry and raw material producers to appropriately treat two commercially relevant types of fabrics with the DWR alternative chemistries (fluorinated and non-fluorinated). We compared the performance of the treated fabrics developed in the project by testing the following properties using industrial standardised methods: general properties, physical properties, DWR properties and stabilities of properties considering relevant stress- parameters. We demonstrated that non-fluorinated alternatives can have a competitive water repellency in comparison to short-chain PFAS substitutes, but that they lack oil repellence. We further estimated possible loss mechanisms for impurities and/or degradation products from DWR-treated fabrics and conducted a hazard assessment for relevant chemicals based on data available in the literature. Our hazard ranking suggests that hydrocarbon-based polymers are the most environmentally benign, followed by silicone- and fluorocarbon-based polymers. Future work will include risk assessment and life cycle assessments (LCA) to estimate long-term advantages and disadvantages of the different DWR-technologies.

TU077
Dietary exposure to PFOS and PFOS precursors of Norwegian population
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Perfluorooctane sulfonate (PFOS) has been a widely used organic pollutant until its restriction in the early 2000s. It was listed in the Stockholm Convention on Persistent Organic Pollutants (POPs) in 2009. Several studies have been published reporting environmental or dietary exposure data, biomonitoring levels and health concerns. Notwithstanding reports that absolute levels of PFOS in human biomonitoring studies are declining; recent published studies of environmental, dietary exposure data and biomonitoring levels, highlight that uncertainties in terms of exposure and metabolism still remain, as the levels are decreasing slower than predictions suggest. To illustrate, indirect exposure to PFOS-precursor compounds followed by *in vivo* metabolism to PFOS has been highlighted as a potentially important source of current levels of PFOS present in biological samples. Current modelling studies estimate such PFOS-precursor exposure after the 3M phase out contributes between 10 and 40% of total PFOS body burdens. This evidence is supported by a small number of *in vivo* and *in vitro* studies, which report conversion rates up to 20% (e.g. EtFOSE in long term exposed rats). As a consequence of this evidence, further study of the external exposure to these PFOS-precursors substances is required. In an effort to enhance understanding of why levels of PFOS are not decreasing as expected, this study reports dietary exposure to the following PFOS-precursor compounds: sulfonamides (FOSAs), sulfonamidoethanols (FOSEs) and sulfonamide alcohols (FOSAAs). This is achieved via analysis of 24 h duplicate diet samples from 61 Norwegian adults in 2014 These data will be linked to food questionnaires, to help identify which groups of food are the main contributors to dietary exposure to these compounds for this cohort.

Alternative approaches to animal testing for ecotoxicity assessments (P)

TU078
Concepts, Tools, and Strategies for Effluent Testing: An International Survey
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Whole effluent testing (also called Direct Toxicity Assessment) remains a critical long-term assessment tool for aquatic environmental protection. Use of animal alternative approaches for wastewater testing is expected to increase as more regulatory authorities routinely require fish and invertebrate tests for effluent assessments or as dischargers seek means to reduce overall animal (fish) use. To address the current state of the science associated with the use of alternative (3R) methods in effluent testing and assessment, a survey was conducted to identify the numerous and varied testing strategies to assess the potential environmental impact of effluents. This survey was aimed at summarizing the breadth of effluent testing approaches that are currently used in various regions and countries. Several questions were asked about the types of regulatory programs used to control the discharge of toxic chemicals, and at what level wastewater discharges are regulated. Specific information on what testing methods and species are used was requested, as well as whether non-animal alternative methodologies are used or considered for effluent/wastewater biological assessments. Finally, several questions inquired about the type of treatment for the various types of dischargers and what, if any, regulatory requirements or regulatory monitoring exists to regulate toxics wastewater. This survey provides the baseline knowledge to assess the role of biological testing for wastewater streams and identify opportunities to use novel strategies in an integrated manner to both optimize testing approaches and reduce reliance on animal tests on a global scale. The results of the survey were one of the cornerstones of the International Workshop titled "Concepts, Tools, and Strategies for Effluent Testing" facilitated by the ILSI Health and Environmental Sciences Institute (HESI) Animal Alternatives in Environmental Risk Assessment Technical Committee. *Disclaimer: The views, conclusions and recommendations expressed in this article are those of the author and do not necessarily represent views or policies of Environment Canada or the US Environmental Protection Agency*

TU079
How the integration of sub-lethal criteria and of genotoxicity assessment can improve the Fish Embryo Toxicity test sensitivity

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The Fish Embryo Toxicity (FET) test using zebrafish (*Danio rerio*) is commonly used as an alternative to determine acute toxicity of chemicals or environmental samples on adult fish. In the FET, only lethality is used to calculate a LC_{50} value after 96h of exposure. This work aims to integrate to the FET 9 sub-lethal events: abnormal eye development and/or pigmentation, presence of edema, blood circulation defect, malformations (head, tail; heart, spine and yolk sac) as well as the DNA damage level in cells isolated from 96h exposed embryos. The objective is to improve the sensitivity of the test and to achieve a more accurate toxicity evaluation of samples. This approach was first tested with a model toxicant: 3.4-dichloroaniline (3.4-DCA) and was then used to assess the residual toxicity of two wastewater effluents (hospital and urban mixture) treated by conventional activated sludge alone (CAS), or followed by tertiary ozonation (CAS+O_3) with an ozone dose of 5 mg O_3/L. Considering dead embryo or alive embryo with at least one of the 9 sub-lethal criteria equally as “affected embryo”, an EC_{50} value of 1.65 mg/L was estimated after 3.4-DCA exposure, when the FET LC_{50} was 3.75 mg/L. In a second approach, the distribution of the number of sub-lethal plus the lethality criteria (0-10) among 60 embryos per concentration was represented as a score. The number of abnormal embryos and the number of sub-lethal criteria recorded per embryo increase with 3.4-DCA concentration. Considering only the mortality endpoint, a significant difference compared to the control was found at 3.7 mg/L, while through the integration of sub-lethal endpoints a significant difference compared to the control was already observed at 0.7 mg/L of 3.4-DCA (Wilcoxon rank sum test). Moreover, a significant increase in the DNA damage level (Comet assay) was found in embryonic cells from the lowest tested 3.4-DCA concentration (0.7 mg/L). Embryo mortality in CAS+O_3 effluent was not statistically different compared to the control but a significant increase in developmental abnormalities was observed. Additionally, a residual genotoxicity was shown in this WWTP effluent despite tertiary ozonation. With the integration in the FET of several sub-lethal criteria and of a genotoxicity assessment, an improved sensitivity of the bioassay was achieved that allowed to reveal a residual toxicity in a treated wastewater characterized by a complex organic chemical mixture at low concentration.

TU080

Environmental Effects Monitoring as a tool for Effluent Testing

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As part of the HESI Animal Alternatives in Environmental Risk Assessment Technical Committee's "Concepts, Tools, and Strategies for Effluent Testing: An International Workshop" various Case studies were presented on methods used in different countries to monitor effluent. In Canada starting in 1992 an Environmental Effects Monitoring (EEM) program was developed for the Pulp and Paper industry. This program requires each discharger to monitor fish and benthic invertebrates both upstream and downstream of its discharge to evaluate effects it may have on the receiving environment. The Government of Canada than uses this information Nationally to determine whether its existing discharge limits for the industry are protective of all receiving environments. The program is cyclical in nature with upcoming cycles dependant on results of the previous cycle on a mill specific basis. Effects in a receiving environment are confirmed in the next cycle, following confirmation the extent and magnitude of the effects are evaluated. Once effects are documented, investigation of cause and solution are then implemented. The pulp and paper program is now entering its 8th cycle and has implemented solutions to some of the effects documented. The EEM field program is then used to confirm improvements in the receiver following implementation. The program was adapted for use at all metal mines in Canada in 2002 and has considered implementation for municipal waste water discharges. It is also the same methodology implemented for the fish program in the Alberta Oil Sands Area. This program has been used or applied in a number of countries including Chile, Brazil and Uruguay. Discussions will include how this tool has been used for effluent testing in Canada and if it may be used as an alternative for laboratory effluent testing in certain cases.

TU081

Implementation of ecotoxicological tests using freshwater planarians: Choice of cultivation medium by using the fecundity mean index as viability marker

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Freshwater planarians have been successfully used in several ecotoxicological methods. This invertebrate can respond to environmental contaminants from molecular to behavioral level. However, there are no standard guidelines for bioassays or laboratorial cultivation. Consequently, there are problems in cultivation and in results replication and interpretation. This work aimed to use the reproductive success of two planarian species in laboratory as deciding factor for the cultivation medium’s choice. Sexual specimens of *Girardia tigrina* (Gt, n=45) and *Schmidtea mediterranea* (Sm, n=20) were donated by the Max Planck Institute for Molecular Biomedicine (Münster, Germany). During the following four weeks of adaptation, all animals were maintained in glass vessels containing the donator’s medium (Münster’s tap water plus Montjuic salts (MJ) – 1.6 mM NaCl, 1 mM $CaCl_2$, 1 mM $MgSO_4$, 0.1 mM $MgCl_2$, 0.1 KCl and 1.2 mM $NaHCO_3$, pH 7), but any cocoons were produced. After that, animals were split in 10 experimental groups according to specie (Gt and Sm) and cultivation medium (Essen’s tap water plus and without MJ, commercial spring water plus and without MJ and ultrapure water plus MJ). The population density was 0.025 org/ml. During the five testing weeks all vessels were held in darkness under controlled room temperature (17.9 ± 0.9°C). After weekly feeding with bovine liver, the medium were completely renewed. To determine the mean fecundity index (FC) the total number of cocoons collected in each experimental group per week was divided by the respective number of planarians. Then, the weekly FC’s of each group were compared using analysis of variance (ANOVA) followed by Tukey test, at significance level $p \leq 0.05$. As a result, the higher and statistically significant FC values were 0.59 ± 0.4 (Gt) and 0.32 ± 0.4 (Sm) obtained in the groups just in tap water. The other groups presented lower FC values ranging from 0 to 0.3. In short, all observed FC’s are still lower than the expected for planarians. As an example, literature indicates a FC > 1 for Gt in healthy laboratorial conditions. However, the fecundity mean index has been shown as a great marker to assess planarian viability in laboratory.

TU082

A Critical Review of Mode of Action (MOA) Assignment Classifications for Ecotoxicology

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There are various structure-based classification schemes to categorize chemicals based on mode of action (MOA) which have been applied for both eco- and human health toxicology. With increasing calls to assess thousands of chemicals, some of which have little available information other than structure, clearly understanding how each of these MOA schemes was devised, what information they are based on, and the limitations of each approach is critical. Several groups are developing low-tier methods to more easily classify or assess chemicals, using approaches such as the ecological threshold of concern (eco-TTC) and chemical-activity. Evaluation of these approaches and determination of their domain of applicability is partly dependent on the MOA classification that is used. The most commonly used MOA classification schemes for ecotoxicology include Verhaar and Russom (included in ASTER), both of which are used to predict acute aquatic toxicity MOA for fish. Verhaar is a QSAR-based system that classifies chemicals into one of 4 classes, with a 5th class specified for those chemicals that are not classified in the other 4. ASTER/Russom includes 8 classifications: narcotics (3 groups), oxidative phosphorylation uncouplers, respiratory inhibitors, electrophiles/proelectrophiles, AChE inhibitors, or CNS seizure agents. Other methodologies include TEST (Toxicity Estimation Software Tool), a computational chemistry-based application that allows prediction to one of 5 broad MOAs (AChE inhibition, narcosis, reactivity, uncoupling, and neurotoxicity), with specific MOAs developed as subcategories. MOAtox is a dataset of MOA assignments for over 1200 chemicals that draws from various schemes and assigns 6 broad and 31 specific acute aquatic toxicity MOAs. Cramer Classification is a widely used approach for classifying and ranking chemicals based on oral systemic toxicity, and is based on short-term or chronic mammalian dietary studies. This scheme is used as the basis for the human health TTC, though its structure-based approach may be useful for ecological applications. Other classification systems that provide insight on a chemicals’ MOA include the Wood Pesticide Compendium, WHO Classification of Pesticides, and other information included in risk assessment tools, such as the USEPA’s EPISuite and the OECD QSAR Toolbox. This presentation will summarize the available MOA assignment schemes, providing a critical evaluation of their limitations and utility.

TU083

Acetylcholinesterase activity, in the orb web spider Agalenatea redii (Scopoli 1763) (Araneae, Araneidae). Characterization and sensitivity to an organophosphate pesticide

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In the European context aiming at reducing the use of pesticides, biological control against pests has to be encouraged and require the protection of beneficial species. Thus studying the effects of pesticide use on current natural enemies is necessary. The spider *Agalenatea redii* is a non-target species, present in apple orchards, and could be a model for both an ecotoxicological and ecological approaches. The objective of this work was to characterize the *A. redii* acetylcholinesterase (AChE) and to assess in laboratory conditions, its sensitivity towards Pyrinex®, an organophosphate insecticide currently used in orchards. We found that AChE is a membrane-bound activity, preferentially located in the cephalothorax, which exhibited a six times higher specific activity than the abdomen (19±5 and 3±0.48 mu.mg^{-1} of protein respectively). This cholinesterase seems to belong to the true acetylcholinesterases as it preferentially hydrolyzed acetylthiocholine (V_{max} = 19±1 mu.mg^{-1}; K_M = 0.124±0,032 mmol.L^{-1}) than butyrylcholine (V_{max} = 10±4 mu.mg^{-1}; K_M = 4,5±3,2 mmol.L^{-1}) and exhibited a strong inhibition for high acetylcholine concentrations. Moreover, the *A. redii* AChE is very sensitive to eserine inhibition (a selective ChE inhibitor) and is selectively inhibited by BW284c51 (a specific inhibitor of AChE) at concentrations in range of 10^{-5} mol.L^{-1}. No inhibition was observed using iso-OMPA, a selective inhibitor of pseudocholinesterase. After one-week exposure to chlorpyrifos-ethyl (Pyrinex®) at 0.33 times the normal application rate, a significant inhibition of AChE activity was observed in the cephalothorax, with a loss of 30% compared to the control. These results suggest that the AChE is a relevant biomarker to study the effect of Pyrinex® on *A. redii* because of its high sensitivity to organophosphate following pesticide exposure. Key words: Acetylcholinesterase ; Spider; Ecotoxicology ; Insecticides

TU084

Stepwise screening scheme for identification of animal testing alternatives

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When seeking for registration of a substance, GLP-guideline studies are normally employed on the substance as test item to obtain the basis for an assessment as required by the applicable guidance. Nonetheless there could be other possibilities to reach a meaningful conclusion. Furthermore a systematic evaluation assures avoidance of test artefacts, which may not only produce unnecessary cost and animal consumption, but also the requirement to repeat a study. Generally the following options should therefore be considered first. 1. Waiving an endpoint - Not assessing on an endpoint information requirement\n A short-term effect study may be unnecessary when long-term exposure results exist. In such case the acute toxicity testing may be omitted. 2. Waiving an experiment - Not testing for an endpoint information requirement\n Apart from the three standard waiving arguments, i.e. testing does not appear scientifically necessary \n because existing (literature) data suffice, testing is technically not possible and exposure based (below adequate Threshold of Toxicological Concern), assessment of one endpoint by data from a different one can be possible: Endpoint-Analogy Read-Across: \n e.g. chronic fish toxicity from a carcinogenicity study using fish (US NTP protocol) 3. Analogy approaches (equimolar basis)\n Experimental evidence can be used from tests with surrogates or analogues: Surrogates: \n Identical chemical species liberated (source chemical) with\nanalogue bioavailability due to degradation kinetics or dissociation “Prodrug” \n target chemical forms source chemical as primary degradant “Converging pathway”\n degradation pathways of source and target chemicals lead to formation of a common degradant known to produce the effect “Actual exposure”\n different but non-toxic and thus irrelevant counterion after dissociation Analogues: \n Identical bioavailability after lipophilicity based correction of\nanalogue chemical species sharing a mode of action One-to-one read-across from only one chemical Trend analysis (QSAR) within a larger group forming a category 4. Mixture effect evaluation and calculations\n In a “prodrug” case, if a substance is cleaved into two new substances whereof one is suitable for read-across, both may contribute to the overall effect. Also known impurities should be assessed using the appropriate model (independent or combined action).

TU085

Structural Alerts to Predict Excess Toxicity in the Pimephales promelas Assay

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Since REACH a large number of chemicals have to been evaluated in terms of risks for environment and human health. Further animal tests should be reduced. In aquatic toxicology, *Pimephales promelas* had been used widely as bioassay to study adverse effects of chemical substances. While the toxicity of narcotic compounds can be predicted reasonably well through simple regression equations employing the octanol/water partition coefficient as hydrophobicity parameter since decades. Even though fish toxicity may be interpolated by a read-across approach within a limited chemical domain [1], there are no corresponding general relationships for compounds with specific or reactive modes of action on a mechanistic base. Taking a dataset of more than 900 organic compounds with experimental values for the 50% lethal concentration within 96 h exposure time (LC_{50}), we have developed structural rules that enable the predictive discrimination between narcotic effect level and excess toxic compounds directly from molecular structure with reasonable results. The discussion includes a comparison with established structural alert schemes for daphnids, other MOA-models for fish toxicity and models predicting general reactivity. So far neither the daphnia structural alert models nor the MOA-models are suitable for use in prediction of excess toxicity in *Pimephales promelas* assay. The new model will be combined with the read-across model [1] in a consensus approach, and will be made publically available in computerised form via the software system ChemProp [2]. Acknowledgement: The SOLUTIONS Project is supported by the 7th Framework Programme (FP7-ENV-2013) of the European Union under grant agreement no. 603437. **References** [1] Schüürmann G, Ebert R-U, Kühne R 2011. Quantitative read-across for predicting the acute fish toxicity of organic compounds. *Environ. Sci. Technol.* 45: 4616-4622. [2] UFZ Department of Ecological Chemistry 2015. ChemProp 6.3\n http://www.ufz.de/index.php?en=6738

TU086

Pitfalls in law-abiding long-term fish toxicity testing instead of short-term to reduce animal consumption

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With respect to animal welfare the legal text of REACH Regulation (EC) No 1907/2006 states in Annex VIII, whose requirements must be submitted with the dossier at the respective dead line, (Table, 9.1.3, column 1): “*Short-term toxicity testing on fish: the registrant may consider long-term toxicity testing instead of short-term*.” This is based on the consideration that the short-term test can be omitted if long-term data are generated directly (without a previous short-term study), thus using less fish. On the other hand the long-term fish study is an Annex IX endpoint for which it is generally mandatory to submit a testing proposal before conducting a study. In consequence it is possible that no fish toxicity data would be available in the submitted dossier thus making it incomplete. In result the choice when following the column 1 recommendation would be to select between non-compliance with regard to data completeness (lack of fish data) \n or with regard to the duty to follow the test-proposal procedure. Another conflict in fish long-term testing exists in the registration of Medicinal Products for Human use (MPH). A long-term fish early-life stage test is part of the aquatic base set requirements, which has to be conducted if the maximum daily dose (DOSEai) exceeds the action limit of 2 mg/patient and day, but the DOSEai may still be reconsidered until final submission. Independent from DOSEai, an assessment for Persistence, Bioaccumulation and Toxicity (PBT assessment) potentially including fish toxicity testing is nonetheless triggered for substances whose lipophilicity exceeds a Log Kow of 4.5. In this PBT assessment the employment of an alternative test for acute fish toxicity is prescribed. When the substance is found not PBT/vPvB or if the risk characterisation ratio is < 1, a long-term fish test must not be performed. The poster presents the details of these regulatory dilemmas. It is considered for guidance purposes only and should not be regarded either as a personal or company recommendation or a substitute for taking legal or other professional advice.

TU087

Statistical Approach to Inform the Study Design of an Inter-laboratory Comparison of In Vitro Methods (S9 Fraction and Isolated Cryopreserved Hepatocytes) to Estimate Hepatic Metabolism in Fish)

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In order to support the development of an OECD Test Guideline (OECD Project 3.13), a multi-laboratory ring-trial is being conducted to evaluate in vitro methods used in assessing chemical biotransformation in fish involving both trout liver subcellular fractions (S9) and cryopreserved hepatocytes. Substrate depletion methods are used to determine the rate at which both test systems metabolize a series of test chemicals. The goal of the ring trial is to compare in vitro S9 and hepatocyte performance across participating laboratories and S9 performance to hepatocyte performance. In order to inform the study design for the current ring trial, we examined the results from previous multi-laboratory studies with multiple chemicals which used trout hepatocytes and S9 fractions to estimate whole-body rates of chemical metabolism. An analysis of the existing data was conducted using a linear-mixed effects (LME) model which was fit with a restricted maximum likelihood estimation procedure to determine the sources of variability in the data and to estimate the depletion rate. This information from the LME model analysis was subsequently used to set Monte Carlo simulations (>1000) varying the parameters that contributed to the variance in the model (number of time points, replications, chemicals, and laboratories) in order to identify the optimal experimental design for the current ring trial. For each simulation we computed the coverage probability of a 95% confidence interval and mean confidence width. The final study design was developed based upon a small mean confidence width and greater coverage probability in order to achieve a statistically robust study while including practical considerations such as cost, availability of biological material, time, and other critical resources.

TU088

Crossing frontiers - using zebrafish and nematode models for both ecological and mammalian toxicity screening purposes

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Under the principles of the 3Rs (reduction, replacement and refinement) there is a need for 'animal' alternative toxicity screening assays to assess potential risks to man and the environment. For human health hazard assessment these screening assays need to be translational to humans, have high throughput capability, and from an animal welfare perspective be harmonized with the principles of the 3Rs. To avoid some of the limitations of cell culture systems (i.e. lack of biotransformation capacity, multicellular or multi organ complexity) we have used *Caenorhabditis elegans* (nematode) and *Danio rerio* (zebrafish) larvae models as alternative assays for developmental and reproductive toxicology (DART) hazard assessment of some candidate chemicals. Both models follow the guidelines on the protection of experimental animals (Council of Europe, Directive 2010/63/EU) and are counted as non-animal tests. In our studies the results of the zebrafish and nematode studies were in alignment with data obtained from conventional mammalian toxicity studies indicating that these have potential as developmental toxicity screens. Although their applicability domain has yet to be established it is apparent that there are a number of conserved genetic pathways across the species. In addition to providing mammalian toxicity screening data we believe that, with careful selection of dosing methods and consideration of exposure mechanisms, these models can also provide relevant information for environmental hazard and risk assessment to both aquatic and terrestrial organisms. By combining both mammalian and ecotoxicity considerations into the test designs these models could provide a unique opportunity for environmental and mammalian toxicologists to interact to develop powerful diagnostic tools for a range of applications. For example, such models could have significant potential in screening product categories (e.g. to assess which products should be selected for higher tiered testing) and in screening of water and groundwater samples. In the latter examples the models could be used to provide a more holistic indication of both health and environmental risks from contaminated waters. The models and potential applications will be discussed in the presentation but we recognise that the true potential of such screens will only be realised if we are not 'shackled' by the boundaries of our classical specialisms and embrace an interdisciplinary approach from the outset.

TU089

Score sheets as a tool to enable consistent and comparable identification of the moribund stage in exposed fish in fish acute toxicity tests

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The OECD Guideline 203, Fish acute toxicity test, is currently under revision by the OECD expert groups. In recent years and particularly with Directive 2010/63/EU taking effect in September 2010 including its requirement "to reduce the duration and intensity of suffering to the animal to the minimum possible and ensure a painless death", there has been on-going discussion how to replace this test method or at least ensure tests are compliant with the growing demands of animal welfare legislation. For this reason, the revision process is especially focused on the stage of moribundity. If this stage can unambiguously identified in an exposed animal it should be removed from the exposure tank and humanly killed to avoid unnecessary suffering. The question, how moribund fish are included in the evaluation of the study endpoints, is part of the expert discussion during guideline revision. One of the concerns to this approach is the high degree of subjectivity in the identification of moribundity, a process which requires experienced staff and clear-cut definition of clinical signs/sub-lethal effects to be assessed. The authors suggest implementing a scoring system, which is a standard requirement during performance of animal tests at least in Germany. In this scoring system, parameters are defined which enable different stages of suffering to be classified. The scale includes the stages of; no suffering, mild suffering, moderate suffering and severe suffering resulting in moribundity and eventually death of the fish. The parameters to be assessed include body coloration, behaviour, swimming position and general appearance. Each parameter is scored based on expression of sub-lethal effects and clinical signs. The sum of scores will induce different actions, which includes; enhanced monitoring, contacting the study director and declaration of moribund stage followed by humane killing. The authors present a harmonised scoring sheet provided by different European fish research laboratories. This presentation is intended to deliver further input into the revision process of OECD guideline 203.

TU090

The use of trout liver S9 fraction and cryopreserved trout hepatocytes in substrate depletion assays for the evaluation of xenobiotic biotransformation by fish.

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Chemical biotransformation represents the single largest source of uncertainty in chemical bioaccumulation assessments for fish. To improve modeled BCF predictions in fish, a substrate depletion assay using trout liver S9 fraction or hepatocytes is a simple means to estimate chemical biotransformation rates. In this approach, a test chemical is incubated with the biological material under first-order kinetic conditions, and the loss of the chemical in the system is measured over time. From the depletion rate, an in vitro intrinsic clearance is calculated, extrapolated to an in vivo clearance, and incorporated in a bioaccumulation model to predict the BCF. A multi-lab ring-trial is underway with the goals of demonstrating the reliability (reproducibility and transferability) of the trout in vitro biotransformation assays, and to support the development of an OECD Test Guideline. Pooled liver S9 fractions and primary hepatocytes were isolated from rainbow trout and characterized using standard substrates to assess both Phase I and II metabolic activity. The material was then provided to six laboratories to perform substrate depletion assays with six chemicals (pyrene, 4-*n*-nonylphenol, cyclohexyl salicylate, fenthion, deltamethrin, and methoxychlor). Incubations were initiated by spiking the test chemical into the reaction mixture in a single test tube, and terminated by pipetting subsamples into a solvent containing an internal standard at pre-determined sampling times. The temperature and pH of the incubation system were the same as the acclimation conditions of the source fish. Preliminary studies were conducted to determine the starting concentration of test article, and the test design was informed by a modeling-based evaluation of hypothetical test data (see companion poster by Embry et al.). These procedures provide a highly standardized test protocol that is optimized for use in comparative biotransformation studies with fish. *The views, conclusions and recommendations expressed in this poster are those of the authors and do not necessarily represent the policies or positions of the European Commission, the OECD, or the United States Environmental Protection Agency.*

TU091

An intestinal fish cell line of rainbow trout (RTgut-GC) for predicting fish acute toxicity of poorly soluble substances

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Various approaches are investigated with the goal to reduce and replace the use of animals for testing of chemical substances. Fish are being used in environmental risk assessment as well as for the testing of industrial effluents. Thereby the Fish Embryo Toxicity test (OECD 236) has already been validated as suitable alternative. Research onto the use of fish cell lines has shown as well to accurately predict fish acute toxicity. Specifically the *in vitro* cytotoxicityassay based on the rainbow trout (*Oncorhynchus mykiss*) gill cell line is suitable for a variety of organic chemicals. Other than the fish gill, the intestine of fish is considered a major route of exposure and uptake of chemicals, especially for persistent and very hydrophobic substances. When present in water, such substances may adsorb to particles that may be taken up by the oral route. We hypothesize that a novel intestinal cell line of rainbow trout (RTgut-GC) is equally able to predict fish acute toxicity for less water soluble compounds. If proven to be true, this approach may be used to test hydrophobic chemicals, such as fragrances, for regulatory purposes and to reduce the use of animals in a laboratory test. The cytotoxicity of selected hydrophobic fragrances towards the RTgut-GC cells was quantified using previously established protocols. The assays are based on the combination of three fluorescent dyes as a measure of metabolic activity, cell membrane integrity and lysosomal membrane integrity. Fragrances were selected to cover a range of physicochemical properties. Specific extraction and quantification methods were developed and were successfully applied for samples from the cytotoxicity assays to quantify truly present chemical concentration. Dose-response curves were calculated based on nominal and measured concentrations and cytotoxicity values were expressed accordingly. Finally, effect concentrations showing 50 % effect (EC_{50} values) were calculated and compared with concentrations showing 50 % death in fish (LC_{50}) from *in vivo* experiments. We were able to show a very good agreement between *in vitro* and *in vivo* values. In summary, the rainbow trout gill cell line (RTgill-W1) assay was successfully transferred to the intestinal cell line from rainbow trout (RTgut-GC) and applied for the testing of hydrophobic organic chemicals. As was previously shown for RTgill-W1, the RTgut-GC produced acute cytotoxicity data that are predictive of the acute fish data.

TU092

Conduction velocity measurements to assess earthworm exposure to insecticides

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Earthworms are important soil organisms. In agricultural soils, they are potentially exposed to many pesticides. There is a current need for reliable and simple biomarkers to assess the effects of pesticides on earthworms. Among these pesticides, insecticides, especially the neurotoxic ones, represent the highest threat to earthworms. Thus evaluation of the nervous activity by studying the nerve conduction velocity (NCV) could be a relevant biomarker. The aim of this study was to evaluate the effects of 2 insecticides, a carbamate (Pirimicarb) and an organophosphate (Lorsban), both inhibitors of acetylcholinesterase (AChE) enzyme activity on the NCV of *Aporrectodea caliginosa* belonging to the genus of earthworms in the family Lumbricidae. NCV of the medial giant fibers was recorded on days 1, 2, 3, 4 and 7 following exposure to 3 insecticide doses (0.5X, 1X, 5X where X is the predicted environmental concentration based on the normal field application rate) and a control. AChE activity of *A. caliginosa* homogenates was measured at the end of the experiment. A significant decrease in NCV was observed with Pirimicarb on days 3, 4 and 7 for doses 1X and 5X. AChE activity was significantly decreased for the same 2 concentrations. For Lorsban, no significant changes were observed. This study shows that NCV could be an interesting new biomarker to study the impact of insecticides on earthworms. This physiological biomarker is easy to perform, cheap, quick, does not harm the earthworm and fills a missing link between other biomarkers.

TU093

Effects of the endocrine disrupter triclosan to early life stages of the marine fish "Solea senegalensis"

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Early life stages of Senegalese sole (*Solea senegalensis* Kaup, 1858) are theoretically suitable organisms for studying endocrine disruption in marine vertebrates, particularly during the thyroid-mediated metamorphosis. Previous works have described such effects by triclosan in vertebrates, an anti-bacterial used in personal care products. Therefore, our aim was to investigate the adverse effects of this chemical to early life stages of *S. senegalensis*. Exposure to triclosan was performed in two experiments. A first group of eggs in gastrula stage was exposed to 5 concentrations (0.30-1.0 mg /L) until 96 hours post fertilization (hpf). The second group was maintained in culture conditions until 13 days after hatching (dah), corresponding to the period immediately before the onset of metamorphosis, and then were exposed to triclosan during 48 h (0.20-0.90 mg/L). Mortality, malformations, lethargy, fish length and behavioral response (response to light stimulus) were recorded. The LC_{50} of larvae exposed to triclosan from the egg stage until 96 hpf was 0.555 ± 0.020 mg/L, while the LC_{50} of 15 dah larvae exposed for 48 h increased to 0.664 ± 0.001 mg/L. Malformations also increased with triclosan concentration in the first experiment ($EC_{50}=0.393\pm0.016$ mg/L) with notochord curvature and overall delayed development recorded in 100 % of larvae at 0.74 mg/L. Besides, lethargy increased with triclosan exposure ($EC_{50}=0.4123\pm0.0154$ mg/L) and a decrease in fish length was recorded at the lower concentrations tested (0.30 and 0.41 mg/L, $p< 0.05$). No delay in metamorphosis was observed in 15 dah larvae exposed to triclosan during 48 h; however, malformations strongly related with metamorphosis progress (namely, abnormal left eye migration and cephalic region development) were present, affecting up to 77 % of larvae at 0.9 mg/L. On the other hand, no adverse effects were observed in fish length, neither in their behavioral response ($p>0.05$). As thyroid hormones have a key role during embryogenesis and metamorphosis, our results suggest that triclosan might be able to disrupt HPT-axis of *S. senegalensis* causing malformations and the impairment of metamorphosis. Further testing will include the expression of thyroid related genes in order to assess the modes of actions of triclosan during early life stages.

TU094

Reducing repeat testing of regulatory vertebrate ecotoxicology studies through a critical assessment of Test Guideline criteria

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In practice a significant number of regulatory ecotoxicology studies do not comply completely with the corresponding OECD Test Guideline in terms of meeting the stated validity criteria and/or other parameters outlined in the guideline. This can result in much uncertainty for the decision makers evaluating the studies, and ultimately leads to requests for repeat testing when it is unclear whether these data are acceptable. Study directors may also decide to repeat studies due to a perception/uncertainty regarding whether regulatory bodies or sponsors will accept the study. In some circumstances, repetition may be completely justified if overall test performance is affected, for example where environmental parameters are not suitable for the test species. However in other situations, deviation(s) from the study may not affect its scientific robustness and thus the overall test performance, but could still result in a) rejection due to non-compliance with, for example, all the validity criteria set out in the relevant Test Guideline, or b) the study only being used as supportive information due to perceived shortcomings. This project, led by an expert steering group of international academics, regulators and industry scientists, will explore the range of validity criteria and other required/recommended parameters within vertebrate ecotoxicology OECD Test Guidelines and other commonly used standard regulatory guidelines. The project will determine which deviation(s) (or magnitude of the deviation) fundamentally undermines study outcomes and overall test performance (hence necessitating repeat studies), and conversely which do not impact on the scientific quality of studies (thus negating the need for their repetition). For each Test Guideline the key validity criteria and other study parameters will be considered in detail. The project ultimately aims to provide robust evidence to support the expansion, where appropriate, of validity and/or other criteria within vertebrate ecotoxicology OECD Test Guidelines, to increase the utility of studies in regulatory decision making and decrease the need for scientifically robust *in vivo* studies to be repeated. Consideration of the criteria within the Test Guidelines that are undertaken most often and for which the majority of repeat testing occurs will be prioritised. This presentation outlines the objectives and proposed strategy to achieve the project aims.

TU095

Phase two of inter-laboratory validation of the Xenopus Embryonic Thyroid Signalling Assay

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The Xenopus Embryonic Thyroid signalling Assay (XETA) was designed as a screening assay to provide information on the potential of a test substance to alter the normal functions of the thyroid system. The XETA provides a rapid (< 72h) way to measure the response of embryonic stage tadpoles to potential thyroid disrupting chemicals, allowing a efficient method for screening thyroid disruptors. In addition to serving as a quick screen for thyroid active chemicals, XETA, could serve as a potential alternative method to the *in vivo* Amphibian Metamorphosis Assay (AMA - OECD TG231).The AMA test is based on the study of the metamorphosis of tadpoles after three weeks of exposure to a given chemical, and includes histological examination of the thyroid gland. XETA could provide an alternative test that can be performed quickly, providing information that would be useful for screening large number of molecules or testing environmental samples that couldn't be stored or sampled in large quantities The objective of the validation study is to establish the relevance of the assay by assessing its sensitivity to detect disruption of the thyroid system by compounds active at different points within the thyroid system. The validation is intended to determine the performance and transferability of the assay across a range of both experienced and naïve laboratories. XETA utilizes free-living X. laevis embryonic-stage animals (stage 45 up to stage 47) in a multi-well format to detect modulation of thyroid receptor signaling by potential thyroid active chemicals. The assay is transcriptional-based, and uses a transgenic tadpole line containing the THbZIP genetic construct to detect the activity of Thyroid active molecules that work through various mechanisms. The phase one ring test experiments gave the expected results for the chemicals chosen. A statistical approach was determined and a great consistency of the results was observed between laboratories. The XETA Phase I results demonstrate that the assay provides reasonable sensitivity with the chemicals tested and is reproducible, with a few exceptions, across replicates and labs. The XETA phase II started in fall 2015 and active chemicals with modes of action that were not covered in phase I are tested. Estradiol was selected as an inert chemical to challenge the assay and the statistical analysis procedure. The XETA protocol has been modified in accordance to lesson-learned during the phase one.

Interpreting Biological Effects of Metals and Their Mixtures (P)

TU096

Toxicity of equitoxic and non-equitoxic zinc and cadmium mixtures to Daphnia magna

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In contaminated ecosystems organisms are generally exposed to mixtures of many chemicals, while toxicity tests are often performed on single substances. In the sparse cases of mixture toxicity testing, almost exclusively equitoxic mixtures are tested in stead of non-equitoxic mixtures. The aim of this study was therefore to compare the toxicity of equitoxic and non-equitoxic mixtures of zinc and cadmium to *Daphnia magna*. To this purpose *Daphnia magna* 48 hour acute immobilisation tests were performed with (non-)equitoxic mixtures of Zn and Cd prepared according to the Toxic Unit concept using separately determined EC_{50} values for Zn and Cd. The EC_{50} values of Zn and Cd were 10177 µg/L and 127 µg/L respectively. The EC_{50} of the equitoxic Zn+Cd mixture was calculated to be 1.09 TU (CL: 0.81-1.38), showing that the equitoxic Zn+Cd mixture was concentration additive. In contrast, the toxicity of the non-equitoxic mixtures of Zn+Cd ranged from more than concentration additive to less than concentration additive, depending on the Zn/Cd ratio. This could be explained by differences in the slope of the concentration response relationships of the individual metals, but this was not the case, since these were 8.4 and 7.6 for Zn and Cd respectively. An alternative explanation is that Zn and Cd possibly compete for binding sites at metallothionein-like proteins. Since non-equitoxic mixtures of Zn+Cd dominated by Cd contained less metals in total (because of the much lower EC_{50} value for Cd compared to Zn), the available metallothionein-like proteins pool could bind a higher part of the Cd dominated mixture than of the Zn dominated non-equitoxic mixtures. Consequently, the daphnids could withstand the Cd dominated non-equitoxic mixtures better than the Zn dominated non-equitoxic mixtures. Given the observed ratio-dependent additivity of Zn and Cd in non-equitoxic mixtures, it is concluded that deviations from equitoxicity may lead to over- or underestimation of the additivity of metals in mixtures.

TU097

Effects of metal mixture contamination on a freshwater community: can predictive mixture toxicity models be validated experimentally?

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In the EU and world-wide, the assessment and management of potential risks posed by chemicals is almost exclusively based on exposure and effect evaluations of individual substances. Previously, four different methods - combining the concentration addition (CA) model or the Independent Action (IA) model with Species Sensitivity Distributions (SSD) in four different ways - were used, compared and critically evaluated for their ability to predict metal mixture toxicity. However, the validity of these predictions needed to be further evaluated using dedicated laboratory and/or field studies. Therefore, the present study assessed the predictive capacity of these models by evaluating potential risks to a freshwater community exposed to metals occurring in ternary-metal (Cu, Zn, Ni) mixtures. For this experiment, natural surface water and a naturally occurring planktonic community were collected from a pond in The Netherlands. These communities were exposed to metal mixtures in which each metal was present (1) at its biotic ligand model (BLM) based, bioavailability-corrected HC_5 and HC_{50} concentration (i.e. the concentration that is hazardous to 5% and 50% of the species in a community, respectively) as well as (2) at ascending concentrations of metals with a fixed environmentally realistic metal concentration ratio of 12.6 for Zn:Cu and 2.6 for Zn:Ni (on a μg/L basis). Different community structure and function aspects including abundances of different planktonic groups and primary production rates were examined and compared to the fraction of Potentially Affected Species (PAF) per water body. The latter was calculated using CA and IA as predictive mixture toxicity models. In this way, we were able to test the accuracy of predictions of metal toxicity to freshwater communities made by these mixture toxicity models and thus contribute to the risk assessment of metal mixtures.

TU098

Evaluation of Toxicity Effects of Heavy Metal Contaminated Soils on Earthworm (Eisenia foetida) in a Mining Area of Guizhou Province

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Typical soils contaminated by multiple heavy metals were collected from a mining area in Guizhou province for 28d chronic toxicity assay. Activity of antioxidant enzymes, i.e. catalase (CAT) and superoxide dismutase (SOD), 8-hydroxy deoxyguanosine (8-OHDG) and metallothionein (MT) in the earthworm (*Eisenia foetida*) were determined to evaluate correlations between these indicators and soil heavy metal contents. Feasibility of using these indicators to assess ecological risk of heavy metal contaminated mining soils was investigated. Results showed that the activity of CAT and SOD in earthworms increased firstly, and were inhibited thereafter during the exposure duration. This might suggest that activity of CAT and SOD in earthworm was raised to remove free radicals caused by stress of heavy metal contamination so as to adapt to changes of the environment. The accumulation of metabolites in earthworms after alleviating of metal toxicity inhibited activity of antioxidant enzymes, which led to decrease in CAT and SOD activity. When exposed to sub-lethal heavy metal concentrations, content of 8-OHDG in earthworms showed significant decrease with exposure time. With increase in exposure concentrations, more serious damages on 8-OHDG were observed. Within the exposure duration of 28 days, MT in earthworm played a detoxification role under the stress of heavy metal contamination. MT contents increased after 7 days of exposure and decrease thereafter. This study performs a comprehensive evaluation on ecological risk of typical heavy metal contaminated soils of a mining area based on toxicity assay. Results of this study will be helpful to guide remediation of heavy metal contaminated soils as well as its reuse after treatment.

TU099

Combined effects of interspecies interaction, temperature and zinc on Daphnia longispina population dynamics

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During the last decades, the earth's climate has changed rapidly by human activity. Several studies have already investigated the effect of global warming on aquatic communities. However, most studies have, so far, focused on the effects of single stressors and ignored species interactions, while in nature, multiple stressors and species interactions are present simultaneously. Predicting and understanding the consequences of global warming and species interactions on the effects of chemicals on aquatic populations and communities are a primary goal of ecotoxicological research. The main objective of this study was to assess the combined effect of temperature, interspecific interactions and zinc (Zn) on a *Daphnia longispina* population. During an indoor microcosm experiment, *D. longispina* populations (5 adults and 5 juveniles) were exposed to 2 different temperatures (natural conditions: 16-19°C, global warming prospective: 21-24°C), 2 levels of interspecific interactions (initial abundances of *Brachionus calyciflorus* of 0 & 2900/L) and 3 different Zn concentrations (background, HC_5: 44 μg/l and HC_{50}: 142 μg/l) and were varied in a full 2x2x3 factorial design. During the experiment general water quality (DOC, DIC, pH, and O_2), and *B. calyciflorus* and *D. longispina* abundances were recorded every 3 days, for a period of 4 weeks. Interactive effects between the different stressors on the endpoints were determined by comparing observed abundance values with values predicted with the independent action model. The significance of the interactions (three-way and two-way) and the main effects were tested by using ANOVA's (three and two-way) on log-transformed observed data. By evaluating the combined and interactive effects of temperature, interspecific interactions and Zn on a *D.longispina population*, it can be concluded that there are significant interactions between both stressors. Temperature had a positive effect on the *D. longispina* population growth in the control and HC_5 treatment, but an opposite effect in the HC_{50} treatment. Initial analyses also suggests that the sensitivity of *D. longispina* populations to Zn was affected by the presence of interspecific competition. Overall, our study provides information that aids the discussion about integrating multiple stressor interactions and ecological interactions in risk assessment of chemicals.

TU100

The effects of zinc on the structure and functioning of a freshwater community: a microcosm experiment

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One of the major problems with risk assessment of chemicals is the extrapolation of laboratory single-species toxicity tests, which oversimplify the actual field situation by ignoring species interactions, to natural populations and communities. Here we test if the $HC_{5\ plankton}$ (bioavailability-normalized HC_5 estimated from chronic planktonic single species toxicity data) for zinc (Zn) is protective for a plankton community and we investigated the direct and indirect effects of both the HC_5 and HC_{50} of Zn on a freshwater community's (zooplankton, phytoplankton and protozoa) structure and function. Microcosms were exposed to 3 different Zn concentrations (background, $HC_{5\ plankton}$: 75 μg Zn/L and $HC_{50\ plankton}$: 300 μg Zn/L) for 5 weeks under controlled environmental conditions. The planktonic groups revealed a consistent $NOEC_{community}$ of 75 μg Zn/L, similar to or higher than the $HC_{5\ plankton}$, thus suggesting the protectiveness of the $HC_{5\ plankton}$ for the plankton-dominated community exposed in this study. At 300 μg Zn/L a significant reduction in cladocerans (direct effect) generally resulted in an increase of rotifers, ciliates and phytoplankton abundances (indirect effect). Additionally, the phytoplankton community shifted in dominance from grazing-resistant to edible species. Contrary to the Species Sensitivity Distribution (SSD) prediction, which identified phytoplankton as the most sensitive species group, only the total chlorophyll and the abundance of 2 phytoplankton species were adversely affected at 300 µg Zn/L, whereas many zooplankton species' abundances were affected. Although the HC_5 estimated from the bioavailability-normalized SSD was overall protective for the plankton community, the SSD was not able to correctly predict the species sensitivity ranking within their community context at the HC_{50}.

TU101

Chrome in gulls: A global overview

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Gulls are birds that are present in most aquatic and terrestrial ecosystems. These birds are excellent bio-indicators of anthropogenic pollution, because they are opportunistic, omnivores and coexist with humans. They are particularly important to assess the ecosystem´s health and can reflect the environmental levels of pollutants. Chrome is an essential trace element which can become toxic depending on dosage. Cr can affect the immune system and cause damage to kidneys and liver. We reviewed the Cr levels (dry weight) in different species of gulls, depending on the biological matrix analyzed and the geographical location. The highest Cr concentrations were reported in *Larus cachinnans* (9.8 μg/g, n=13), whereas the lowest levels were in *Larus hartlaubii* (0.0013 μg/g, n=10). The highest Cr concentrations were reported in excreta (9.8 μg/g, n=13), and the lowest in the gallbladder (0.24 μg/g, n=5). With regard to the geographic location, the higher and lower Cr concentrations were reported in the Southern Hemisphere (1.54 ± 1.53 μg/g, n=61) and in the Northern Hemisphere (1.15 ± 1.56 μg/g, n = 1842), respectively. According to metal distribution in gull matrices, the Cd levels are as follows: excreta > stomach contents > bones > lung > blood > skin > salt gland > gonads > pancreas > intestine > subcutaneous fat > muscle > kidney > liver > uropygial gland > bones > stomach > eggs > brain > heart > gallbladder. High Cr levels in excreta may correspond to detoxification processes. Since most of the data at global scale are from Northern Hemisphere, future studies should be conducted in gulls from Southern Hemisphere. The droppings of gulls can be a good biological matrix for monitoring Cr. Standardized methodology for Cr detection in excreta is quite needed in order to compare data at global scale. Keywords: Chrome, gulls, pollution, birds. Acknowledgements: Winfred E. Espejo is scholarship CONICYT-Chile for PhD studies. This study was financially supported by FONDECYT-Chile 1140466 granted to R. Barra.

TU102

The physiological modes of action of different metals in Tubifex tubifex.

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Chemical stress induced by metals alters the energetic parameters of the organisms (e.g. decreasing assimilation, increasing costs of maintenance, growth, or reproduction, or posing a direct hazard to the embryo) and in turn some measurable quantity such as reproductive output and/or growth. In this study, the freshwater oligochaete *Tubifex tubifex* was exposed to metal spiked sediments at increasing concentrations of cadmium, copper and nickel (separately). During the 28-day exposure, lethal and sublethal effect parameters were recorded over time (number of adults, juveniles, embryos, production of full and empty cocoons) to construct survival, growth and reproduction time-course patterns. Metal bioaccumulation was also quantified over time, including the subcellular metal partitioning for the characterization of the metabolically available and metal detoxified fractions. Physicochemical variables (salinity, conductivity, ammonia, temperature and pH) were measured for quality control and to determine the relationship between different exposure routes (e.g. particulate and dissolved exposure) and the biological variables. The generation of life-history trait time series (survival, growth and reproduction over time) helps to understand the organism level responses to metal toxicity. These responses represent changes in energy allocation patterns under metal stress and so reveal the physiological modes of action of the metals.

TU103

Combined effect of airborne heavy metals on A549 human lung cancer cells

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Many studies have demonstrated that airborne heavy metals affect adverse effects on human health and are an important key factor of cytotoxicity. Moreover, these heavy metals exist as a mixture in atmospheric environment. However, little investigation was conducted on toxicological study of metal mixture from atmospheric fine particulate matter. The objective of this study was to predict combined effects of airborne heavy metals on in-vitro human cells and obtain a suitable mixture toxicity model. The top three toxic heavy metals (e.g., arsenic, nickel and lead) were selected for mixtures exposed to human alveolar epithelium cells (A549) which were appropriate to testing by inhalation toxicity. Cell proliferation (WST-1), glutathione (GSH), and interleukin (IL)-8 inhibition were observed and applied to the prediction models of mixture toxicity, concentration addition (CA) and independent action (IA) model. For total mixture concentrations, we used half-inhibitory concentration (IC50)-fixed ratio and IC10-fixed ratio of individual toxicity. Results showed that mixture of IC50-fixed ratio was different with IA and CA model in mortality and GSH-relative inhibition. Mixture of IC10-fixed ratio was suitable for mixture toxicity models because of more realistic concentration. IA model (α=0.05, p=0.217) indicated a better agreement with the observed results than CA model (p=0.015) in mortality, indicating dissimilar modes of action. For GSH inhibition, the result indicated a high relevance between observation and prediction by IA (p=0.801) and CA (p=0.360). Meanwhile, IL-8 results were maintained stable and no significant change in immune reaction related to inflammation. In conclusion, biological endpoints are useful with in-vitro tool for estimating toxicity of heavy metals mixture. IA model is rapid prediction model in airborne heavy metal mixture that is combined based on low effect ratio for human health effect.

TU104

Modulatory toxicity effects of metal mixtures on chemosensation and mortality of C. elegans

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The number of anthropogenic sources of heavy metals in soil increased the past several decades. This accumulation creates serious health hazards for diverse animals including humans, hereby remaining a persistent (eco)toxicological concern. While toxic effects of single metals have already been documented under laboratory conditions, very little is known about their interactions and putative additive effects, which occur in the environment. The effects of metals in mixture can be additive, antagonistic or synergistic, and by interacting with each other, metals can severely increase the toxicity. One of the major challenges in ecotoxicology is thus to obtain insights in mixture toxicology to set realistic environmental quality criteria. It is well-recognized that metals can alter neuronal excitability, that they are implicated in neurodegenerative diseases and that they can impair chemosensation. We therefore aim to investigate whether and how metal toxicities modulate the survival rate and (chemo)sensory capacities and how this is translated to the behavioural/organismal level . To do so, we will fully exploit the benefits of *Caenorhabditis elegans* as a unique model for both fundamental neuroscience and (eco)toxicology. Fed adult nematodes will be exposed to copper and cadmium (as single metal and in combination) for 2h, 8h, 24h and 48h to different concentrations.

TU105

Bioaccumulation of elements in brown crabs (Canser pagurus) in relation to salmon aquaculture farming

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The worldwide demand for seafood has increased over the past decades, and will most likely increase further in the future. A large amount of the increased demand has been met through marine aquaculture. However, it is possible that surplus undigested or excreted toxic elements originating from aquaculture feed may be bioaccumulated by other important marine human consumption marine resources. The brown (edible) crab (*Cancer pagurus*) is an important marine nutritional resource, and high concentrations of some toxic elements, especially cadmium

(Cd) has been reported in this human food item. Due to trace contents of toxic elements, such as Cd, in aquaculture feed, or due to high excretion rates of toxic elements in farmed salmons, levels of toxic elements may be high in brown crabs that feed in the vicinity of salmon aquaculture farms in Norway. Thus, the aim of the present study was to compare levels of elements in brown crabs caught close (ca. 300 m) to a salmon farm and in a reference area far from a salmon farm (> 3000 m). Brown crabs were collected in the autumn during 2012-2015 at Mausund, Norway, and analyzed for element concentrations in the edible meat. Sea floor sediments were used for time integrated chemical characterization of the crabs' feeding ground, and samples from the two locations were analyzed for the same suite of elements as the crabs. The study showed that in general, there were no differences in the concentrations of elements (B, Se, Cd, Sn, Cs, Hg, Pb, Al, Cr, Mn, Fe, Ni, Cu, Zn and As) in crabs from the two locations. Thus, salmon farming did not appear to affect levels of toxic element in brown crabs caught in the vicinity of salmon farms. However, relatively high levels of Cd were found in some of the crabs, irrespectively of location.

TU106

Nickel stress induces enzymatic and ultrastructural changes in the green microalga Ankistrodesmus falcatus. Implications for Ni regulation

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Nickel is one of the main toxic pollutants discharged to the environment, at increasing concentrations in the last years due largely to industrial and mining activities. For example, in Mexico, water concentrations can be as high as 1.16 mg L^{-1}. Statistics reveal that Ni production worldwide is about 1.589 million tonnes; among the products that contain nickel are stainless steel, coins, and batteries; for some of these products Ni cannot be substituted with other metals. Phytoplankton is an important community because it acts as primary producers, responsible of organic carbon synthesis and oxygen production (through photosynthesis), being the basis of all the trophic webs in aquatic environments. Nickel is an essential micronutrient that plays an important role in cellular physiology in photosynthetic organisms, but it is toxic at concentrations higher than that required. For this reason, it is important to know how Ni affects the green microalga *A. falcatus* at an enzymatic level and in its ultrastructure when exposed to toxic Ni concentrations. In this study *A. falcatus* was exposed to different Ni^{2+} concentrations, under continuous illumination, at 27 °C during 96 h to determine the IC_{50} value. Considering the IC_{50} value as basis, five concentrations were selected to make the subchronic assays: 1, 4, 5, 8, and 17 μg L^{-1}. Cell density was quantified daily and, at the end of the assay, the activity of catalase, glutathione peroxidase, and superoxide dismutase was measured; scanning (SEM) and transmission electron microscopy (TEM) observations were also done. *A. falcatus* was very sensitive to low concentrations of nickel, being the IC_{50} value 17 μg L^{-1}. Additionally, catalase and glutathione activity increased when this alga was exposed to increasing Ni^{2+} sublethal concentrations. Moreover, SEM images demonstrated morphological alterations observing widened cells with abnormalities. TEM analysis revealed that nickel induced ultrastructural changes including an increased number of polyphosphate granules, reduction in the starch grains number, as well as anomalies in chloroplasts. At the lowest Ni concentration, significant damage in *A. falcatus* was observed; this concentration was much lower than the one established by the Mexican regulation as the allowable Ni content in wastewater discharges (4 mg L^{-1}, average monthly). Results here obtained point out the need to review and modify the permissible limit to avoid toxic effects in phytoplankters.

TU107

Chronic metal mixture toxicity to Ceriodaphnia dubia: a meta-analysis and implementation in ecological risk assessment

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Although metals in the aquatic environment mostly occur as mixtures, ecological risk assessment procedures are currently still based on a metal-by-metal approach. However, before metal mixture toxicity can be integrated into risk assessment frameworks, better knowledge on mixture toxicity effects during chronic exposure is needed. In several experiments conducted over the past years, we investigated chronic toxicity of Ni, Zn, Pb, Cu and Cd mixtures to *Ceriodaphnia dubia* in 6 different binary, ternary and quaternary combinations comprising in total 210 mixture treatments. In the present study, we combined all our mixture toxicity data in a meta-analysis to evaluate the following three risk assessment related questions: I) are mixture effects important?; II) is the concentration addition (CA) model a conservative model for mixture toxicity?, III) which of the two commonly applied model mixture reference models (CA or independent action (IA)) describes metal mixture toxicity most accurately? Overall, the mixture effect was significantly different from the effect of the most toxic metal. This indicates that mixture effects are important, and that the current risks evaluation approaches based on a metal-by-metal approach might not be protective for communities exposed to metal mixtures. In general, the CA model was the most conservative model. Additionally, very few (less than 1%) significant synergistic interactions relative to the CA model were observed. The latter confirms that the CA reference model can be used as a conservative first tier in a tiered metal mixture risk evaluation scheme. Overall, the IA model described metal mixture toxicity to *C. dubia* more accurately than the CA model. Finally, a possible tiered approach is presented.

TU108

Mechanism of Heavy Metals Toxicity and Adaptation at Different Levels of Biological Organization in White Sea Starfish Asterias Rubens L.

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The main purpose of this work is estimation sublethal effects of individuals and mixture heavy metals at different levels of biological organization on the White Sea starfish *Asterias rubens* L. Methods:At cellular level were estimated coelomic cells in exposure experiments *in vitro* and *in vivo*. Coelomocyte primary cell cultures was used to analyzed enzyme activity by photometric methods (NNR-assay) and gene expression by real-time PCR. At organism level were estimated starfish survival and behavior reaction in exposure experiments as well as metal bioconcentration and bioaccumulation in the all body without dissection. At population level were estimated size structure and abundance in points with heavy metals concentration differences. At community level was estimated association symbiotic fauna in different points also. Some of results: Studied area near White Sea Biological Station of Lomonosov MSU characterized by gradient of metal pollution especially iron contamination. Metal bioaccumulation in starfish was not related to the level of contamination of the environment for all investigated metals Metals can be divided into two groups accumulated in the starfish body (Cu, Zn, Cd, Hg), and does not accumulated (Mg, Fe, Ni, Pb). Two starfish color type was investigated that influence on bioaccumulation. Micronuclei induction related to the level of environment concentration of Ni, Cu, Zn, Pb. Heavy metal bioaccumulation and bioconcentration were compared. Bioconcentration has been observed for all investigated metals, except iron. Experiments showed that Cd and Pb can efficiently bioconcentrate and related to the metal concentration in water. Exposure experiments showed starfish high resistance to heavy metals, the most toxic to the starfish appeared copper. The symbiotic association of starfishes *Asterias rubens* and copepods *Scottomyzon gibberum* Scott even in small areas have differences in the populations that may be associated with both the levels of anthropogenic load, and the features of biotopes. The increased number of copepods thus leads to the activation of immune processes with the increase in cell number in the coelomic fluid of the starfish in the most populated areas. At the same time, these processes may greatly be affected by both the natural features of biotopes (salinity, depth, current speed and the type of biotope) and the anthropogenic factor (heavy metals).

TU109

Effect of thermal prehistory and regime on metal toxicity in the zebrafish (Danio rerio)

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The zebrafish has become a valuable vertebrate model organism in a wide range of biological research including ecotoxicology. Although there are several studies on metal toxicity in zebrafish, the effect of thermal background on metal toxicity is poorly documented in this and other model species. Changes in environmental temperature can profoundly change species physiological condition in different ways. In this study we explore whether different temperature regimes affect the organism tolerance against metal toxicity. For this purpose, we defined two main thermal scenarios: **1**.**Temparature shock:** in which fish were exposed to 5 different temperatures (17, 22, 25, 32 and 34 °C) and subsequently exposed to metal spiked waters with Cu, Cd or Cu+ Cd for 10 days. **2. Acclimation:** in which fish were acclimatized to 5 different temperatures for 28 days and then challenged by the same metals or mixtures for another 10 days. The condition of the fish was followed during the experiment, including behavioral and physiological metrics and also water quality was monitored. At the end of the exposure period, whole body metal and major cation (Na, K, Ca and Mg) concentrations were determined using ICP-OES or MS. The results showed that copper was much more toxic than cadmium towards the zebrafish in the medium hard water. However, copper and cadmium together showed a large synergistic effect in the mixture exposures. Fish which experienced a cold temperature challenge had a significantly better tolerance against single and mixture metal toxicity compared to the ones that endured an increased temperature challenge. In single exposures the total body burden of Cu and Cd was significantly higher in high temperature treated fish in comparison to the cold treated ones, but total body burdens is not a good predictor of effect. Analysis of the major electrolytes composition of the body shows that survival strongly depends on the capacity to maintain Na balance. Such an effect was not observed for the other major cations under the metal stress scenarios. The results of these experiments show that the thermal prehistory and temperature

regime plays an important role in determining the tolerance of zebrafish towards the effects of metal exposure.

TU110
Acute toxicity of copper and zinc mixtures to the tropical freshwater cladoceran Macrothrix flabelligera.
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Mixtures of metals (such as copper, lead, zinc, etc.) and organic compounds are frequently found in industrial and municipal wastewaters, as well as in urban storm waters. All of these contaminant sources reach the aquatic receiving systems directly or indirectly, but, considering toxicological aspects, the water quality guidelines are generally obtained from acute and chronic bioassays testing individual compounds. Thus, such criteria can't predict the interactions and effects of the compounds associated in mixtures, which is the most plausible way to find them in the environment. In an attempt to add some new knowledge to this scientific gap, the acute toxicity of copper (Cu) and zinc (Zn) was determined for the tropical freshwater cladoceran *Macrothrix flabelligera* and the association of both metals were investigated through ToxCalcMix analysis. For this purpose, the neonates were exposed to five concentrations of $CuSO_4$ (0.78 to 3.00 µg L^{-1}) and $ZnSO_4$ (27.9 to 79.4 µg $L^{-}1$) for 48 hours. The tests were replicated five times. Results from these acute tests were subjected to probit analysis using the PriProbit Program V1.6.3 to determine EC_{50} values. The average for the EC_{50}-48h/Cu was 1.4 µg L^{-1} and EC_{50}-48h/Zn was 46.5 µg L^{-1}. Binary combinations of Cu+Zn had a significant effect on survival of *Macrothrix flabelligera* and this interaction was classified as additive, indicating the enhancement of adverse effects of mixtures of metals compared to the single metals. The results proved the importance of considering chemical interactions in water quality management. In addition, considering the need of searching for sensitive organisms to represent the realities of different ecosystems to achieve a greater success in water resources management, *Macrothrix flabelligera* can be suggested as a potential test organism for tropical studies.

TU111
Ultrastructural effects of chronic exposure to Pb and Cd in hepatocytes of wood mice, Apodemus sylvaticus
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Lead (Pb) and cadmium (Cd) are two non-essential trace metals (TMs) widely distributed in the environment due to natural sources and anthropogenic activities. They can induce toxic effects in target tissues and cells such as hepatocytes. High resolution electron microscopy, namely transmission electron microscopy (TEM) and scanning electron microscopy (SEM), offers powerful techniques to detect the toxic effects produced by chronic exposure to TMs, from atoms and macromolecules to entire cells. In the present study, we have evaluated the ultrastructural damage of chronic exposure to Pb and Cd in hepatocytes of wood mice, *Apodemus sylvaticus*, inhabiting along a gradient of pollution in the surroundings of the former Pb and zinc smelter of Metaleurop Nord (Nord-Pas-de-Calais, France), combining qualitative and quantitative approaches for TEM and SEM. These ultrastructural parameters have been compared with TMs levels in liver of 64 wood mice live-trapped around the former smelter in 2011. The parameters quantified as biomarkers of hepatocyte damage for TEM and SEM are: localization, ultrastructure, and amounts of α-glycogen, ultrastructural damage and electrodensity matrix of mitochondria, subcellular localization of Pb and Cd, and immunolocalization of matrix metalloproteinases (MMP2 and MMP9). Our results show an increase of all parameters related with cell damage and degeneration in relation to the gradient of pollution. Interestingly, α-glycogen accumulates in relation to Pb levels, whereas mitochondrial damage relates to Cd levels; Pb accumulated in α-glycogen and inclusions of Cd were observed in damaged mitochondria; and heavier localization of cytoplasmic MMPs was found in hepatocytes of mice from polluted sites. These findings indicate the necessity for an effort to study physiological effects of metal pollution at real world conditions in selected species that had previously demonstrated their suitability as bioindicators such as the wood mouse. Then, imaging of ultrastructural effects of chronic exposure to metals offers crucial biomarkers to understand toxic effects in natural populations exposed to anthropogenic stressors such as TMs.

TU112
Comparison of Cadmium accumulation and subcellular partitioning between two marine fish species.
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Kinetics of accumulation and elimination in liver and muscle of two marine fish species, Dicentrarchus labrax, and Solea senegalensis, dietary exposed to 3.5 ppm dw of Cd are investigated. During 60 days, fish were fed with a prepared diet containing Cd, after which a 30 day depuration period was carried out. No significant impact of Cd on growth occurred; however, significant accumulation of Cd occurred in contaminated fish liver and muscle for both species when compared to unexposed controls, with higher levels of accumulation observed in D. labrax. Concerning Cd depuration, 30 days seems to be a too short period to observe elimination in liver and muscle. Quantification of metallothioneins (MT) in liver by spectrophotometry was carried out. Results show that basal levels in D. labrax liver are higher than in S. senegalensis, although exposure dose seems to be too low to induce MT synthesis. Subcellular partitioning of Cd in liver was also investigated, and results show two very different patterns of metal intracellular repartition in these marine species: D. labrax accumulates more Cd in detoxified fractions (about 50%) than S. senegalensis which accumulates Cd in sensitive fractions (about 90%). Our results show a differential accumulation and repartition of Cd between two marine fish species, and underline variability of interspecific sensitivity in metal contamination.

TU113
Bioaccumulation of manganese in an endangered carnivorous marsupial: How does manganese exposure from mining operations affect the health of northern quoll (Dasyurus hallucatus) on Groote Island?
A.b. Amir Abdul Nasir, School of Biological Sciences; F.A. von Hippel, University of Alaska Anchorage / Department of Biological Science; R. Wilson, University of Queensland / School of Biological Sciences
Manganese (Mn) is an element vital for normal cellular processes in all living organisms but deleterious in elevated concentrations. Over-accumulation of Mn in the brain region has been reported to potentially cause a condition known as 'manganism', a neurodegenerative disorder characterized by a range of motor deficiencies, psychiatric disturbances and cognitive problems. However, little is known on how prolonged exposure to lower, atmospheric levels of Mn affects human and whether such exposure impacts natural wildlife populations. To address this knowledge gap, we explored the effect of environmental Mn exposure derived from a large Mn mine on the health of a population of northern quolls (*Dasyurus hallucatus*) on Groote Eylandt. Located in the Northern Territory, Australia, this island has a major Mn mining operation since 1967. In our study, we measured how proximity to the mine was associated with the level of Mn in the environment and the level of Mn accumulated in the hair and organs of the northern quoll. In addition, we are examining how the level of exposure of northern quolls to environmental Mn is associated with (i) motor control deficits, and (ii) altered cortisol level; both of which would indicate long-term health problems from Mn over-exposure. As northern quolls are both terrestrial and semi-arboreal, and thus must consistently traverse variable terrain in complex environments to hunt for prey and find mates, locomotion capabilities are direct measures of fitness and thus relate to the viability of the species on the island. Measuring cortisol level also has health implications for wildlife and humans since elevated cortisol levels are associated with many deleterious endpoints, such as reduced immune response and various disease states. The proximity to the mine was associated with the level of Mn accumulated in the hair and some organs in northern quolls. As Mn levels in hair are shown to be predictive of levels in these organs, so hair is a valid tissue to use for low impact studies (e.g. endangered species). Mn level also had a significant reverse correlation with cortisol level in breeding season, suggesting compromised immunity and reproduction for affected quolls. We demonstrate that ongoing research and monitoring will aid in understanding the costs associated with prolonged, low-level Mn exposure to wildlife health.

TU114
Assessment of the toxicity of metal mixtures in microalgae Monoraphidium sp.
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In this study, an evaluation the effects of metals mixtures: Cd + Cr, Cd + Cu, Cd + Hg, Cd + Mn, Cd + Ni, Cd + Pb, Cd + V, Cd + Zn and Cd + Cr + Cu + Hg +Mn + Ni + Pb + V + Zn in the microalgae *Monoraphidium* sp. was carried. Bioassays were performed with duration of 72 hours to determine the EC_{50}. Subsequently tests with duration of 8 days in which the microalgae were exposed to two sublethal concentrations (EC_{15} and EC_1) were performed to evaluate five biomarkers: Population growth, concentration of chlorophyll, carotene production, production of phenols and lipoperoxidation. The toxicity of metals based on EC_{50} values were (from highest to lowest toxicity): Cu > Cr > Hg > Cd > Zn > Ni > Mn > Pb > V . In bioassays with mixtures of metals was observed that the most harmful combinations was Cd + Cu and the mixture of all metals, agree toxic units calculated. The type of interaction observed in these mixtures was potentiation, with a value of magnification of the effect of 2.3 and 2.5 respectively. In bioassays with mixtures Cd + Cu and Cd + Ni, was observed a decrease in chlorophyll levels. In tests with all mixtures it was detected increased levels of carotenoids and phenols. The highest levels of lipid peroxidation were obtained in bioassays with Cd + Cu + and Cd +Zn mixtures. Because EC_{50} values for Cu, Cr, Ni and Zn metal are lower than those established by the NOM 001-SEMARNAT for discharges in aquatic systems, it is important to continue research and monitoring for responses that indicate the possible damage this microalgae populations by the

action of the discharges and different tensions to prevent irreversible deterioration of the populations in the medium and long term.

Amphipods as models to investigate toxicology of environmental contaminants at the land-sea interface (P)

TU115

Development of assays to determine anxiety-like behaviours in crustaceans.

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Concerns over psycotrophic drugs found in the aquatic environment have necessitated the development of behavioural assays to determine the effects and risk posed by these compounds. Biogenic amines such as serotonin, dopamine and epinephrine are known to control complex behaviours in crustaceans including aggression, movement, and phototaxis. However, it is often difficult to differentiate these without carefully designed experiments. For example, negative phototaxis can be interpreted as scototaxis and positive phototaxis can be interpreted as anxiety-like behaviours. Some recent studies have suggested that invertebrates might be highly sensitive to antidepressants, such as the Selective Serotonin Re-uptake Inhibitors (SSRIs), the Serotonin-Norepinephrine Re-uptake Inhibitors (SNRIs) and Serotonin Antagonist and Re-uptake Inhibitors (SARIs). These drugs alter neurotransmitters in the synaptic cleft by inhibiting its re-uptake into the presynaptic cell, which result in increased stimulation but are also known to effect multiple receptor targets. This ongoing study is currently analysing the effects of a variety of antidepressants on complex behaviours in model crustaceans. Specimens of the amphipod, *Echinogammarus marinus* were exposed to the most prescribed SSRIs (citalopram, sertraline and fluoxetine), SARI (trazodone) and SNRI (duloxetine) at environmentally relevant concentrations from 0.001 to 1 µg/L during acute (1 hour and 1 day) and chronic (8 day) exposures. The movement of the amphipods was tracked using the DanioVision system with EthoVision software for behavioural analysis during 12min alternating dark/light cycles. Results show that light had a significant effect on velocity ($P< 0.001$) indicating escape-related behaviour. Antidepressant concentrations had a significant effect ($P< 0.01$) on velocity for Duloxetine (1hr, 1day & 8days); Sertraline (1hr & 1day) and Fluoxetine (1day); no significant effects were observed for citalopram and trazodone ($P>0.05$). Analyses are ongoing to further differentiate scototaxis, phototaxis, anxiety-like and escape response behaviours. Preliminary results indicate fluoxetine does not influence use of space in a 6-well plate system at any of the exposure concentrations. However, we are currently investigating whether this result may be due to experimental design and test chamber space available to the test organism. These results may have implications for future study design of these types of experiments.

TU116

Frequency of intersex phenotypes of Gammarus pulex (Amphipoda) upstream and downstream from effluent of sewage treatment works.

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There is unequivocal evidence from around the world that sewage treatment effluent has the capacity to cause reproductive endocrine disruption in many fish species. However, the capacity of these effluents to cause reproductive aberrations in invertebrates communities is less clear. This study aimed to determine the sex ratios, intersex frequency and fecundity of *Gammarus pulex* around two sewage treatment works (STW) of similar output discharging into two chalk rivers (the Test and the Anton) in the UK. *G. pulex* were collected at approximately bi-monthly intervals from several points upstream, downstream, and at the point of effluent discharge. *Gammarus* were measured and categorised as male, female, male intersex and female intersex and embryos were counted from ovigarous females. Greater incidences of intersex were observed downstream of the WTP although only significantly ($p < 0.05$) on the river Test. Though the overall incidences of intersex were quite low, they increased by two to three times at and below the effluent discharge point (~1% upstream and 3% downstream respectively) at both sites. The proportion of males and females that were intersex also increased significantly ($p < 0.05$) around the discharge point (~3.1% and 4.1% respectively) compared to upstream (~0.7% and 2.1% respectively). Unlike other similar studies, there was no difference in the male:female ratio seen between the sample sites, however, there was a significant difference in the sex ratios seen between the rivers. The Anton had a much greater difference in the percentage of males versus females (36.7% and 60.2% respectively, compared to 48.8 and 48.9% in the Test). Fecundity varied between sample points but at both rivers it was significantly higher at or near the discharge point (~1egg mg^{-1} female BW) compared to the upstream site (~0.8 mg^{-1}). In other studies, STW effluent has been associated with increases in the fecundity and frequency of female *Gammarus* as well as changes in ovarian development. These changes have been associated with elevated oestrogenic compounds. To date there have been no reports of elevated intersex frequency in amphipods associated with STW, however, amphipod intersex frequency has been shown to increase around sites impacted by industrial pollution. Suggested causal mechanisms include contaminants either feminising the animals directly, or indirectly through immunosuppression and associated increases in feminising parasites.

TU117

The use of Gammarus embryo in ecotoxicology: Effects of cadmium exposure during embryogenesis in Gammarus fossarum.

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In animals, the embryo stage could be more susceptible to a toxic exposure than later stages. Nowadays, assays involving vertebrate's embryos, such as fish and frog embryos, are extensively used. Nevertheless, for invertebrates such studies remained rare. The toxicity of environmental compounds in early life stages is of importance because embryo toxicity has been shown directly implicated in population decline. The aim of the present study is to develop an ecotoxicological assay in embryo of *Gammarus fossarum*. *Gammarus fossarum* is an amphipod widely distributed in European freshwater systems. As it contributes to the degradation of the organic matter and it serves as food for many macroinvertebrates, fishes and amphibians, it plays a major role in the food web. After fertilization, embryos were gently removed from the females and directly exposed to increasing cadmium (Cd) concentrations (0 µg/l, 2 µg/l and 4 µg/l) until they hatched. Cadmium is a non-essential metal widely detected in freshwater ecosystems. *Gammarus spp*. are particularly sensitive to this metal. Daily, the mortality was recorded. After hatching, we measured in the neonates: i) the mass, ii) the locomotor activity and iii) the total phenoloxidase activity (both prophenoloxidase and phenoloxidase activities). In arthropods, phenoloxidase is an enzyme involved in the sclerotization of the exoskeleton, the melanization and the immunological response. By comparison with the control group, we observed a slight – but not significant – increase of the mortality during the hatching period in the group exposed to 4 µg/l. The mass of the neonates was not significantly altered by Cd exposure, whereas the locomotor activity was significantly reduced in the group exposed to 4 µg/l. Surprisingly, with increasing Cd concentrations, the prophenoloxidase (an inactive precursor of phenoloxidases) activity was increased whereas the phenoloxidase activity was decreased. These results suggested that embryo of *Gammarus fossarum* might be used as a sensitive life stage in ecotoxicological studies.

TU118

UV irradiation and leaching reduce adverse effects of imidacloprid contaminated leaves for the shredder Gammarus fossarum

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In recent years neonicotinoid pesticides have become one of the most widely used class of insecticides worldwide in agriculture, horticulture, forestry, and tree nursery. One reason for their success is their systemic nature leading to a fast uptake and distribution of these insecticides within treated plants. However, due to their environmental persistence within plants (up to several months), neonicotinoids may enter adjacent surface waters together with the treated plant material (e.g. contaminated leaf litter). There, neonicotinoids may pose a threat to non-target macroinvertebrates, which feed on these leaves (e.g. shredders), and to associated ecosystem functions, such as the breakdown of leaf litter. However, prior to the consumption by shredders, neonicotinoid contaminated leaves may be exposed to sunlight (i.e. ultraviolet irradiation; UV) and experience the loss of water soluble substances during leaching, which might alter the leaves' internal neonicotinoid concentrations. To assess alterations in the toxicity of the photolabile and water soluble neonicotinoid imidacloprid within leaves we used the key leaf-shredding macroinvertebrate *Gammarus fossarum* (Amphipoda) as test species, while its feeding rate over seven days was recorded as ecotoxicological response variable. In two independent experiments, *Gammarus* was exposed to leaves from imidacloprid-treated (0.15 g active ingredient per cm trunk diameter at breast height; soil applied) black alder (*Alnus glutinosa*) trees which were either a) leached in water (for 1, 3 and 7 days) or b) irradiated with UV light (for 24 h at UV-A: 41 W/m^2 and UV-B: 0.15 W/m^2; in addition to 1 day leaching in water) prior to use in the experiment. Contaminated leaves without prior UV or leaching treatment as well as leaves from untreated trees served as positive and negative control, respectively. Both experiments revealed a by ~70% reduced feeding rate of *Gammarus* when exposed to contaminated leaves without prior treatment compared to the uncontaminated negative control. In contrast, treatment of leaves with UV-irradiation (24 h) and leaching (7 days) completely reduced the adverse effects on gammarids' feeding. Therefore, although consumption of neonicotinoid-contaminated plant material adversely affects leaf-shredding organisms and, hence, leaf litter breakdown, results of this study suggest a mitigating effect of UV light exposure and dwelling time in water.

TU119

Development of a SRM-based multiplexed quantitation of protein biomarkers in Gammarus fossarum

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Biomarkers are recognized as relevant tools for diagnostic and hazard assessment of aquatic systems. However, few are used in invertebrate species studies. It mainly results from the lack of genomic and/or proteomic data, preventing the development of specific and direct method for the quantification of biomarkers. Recently, a novel proteogenomics approach has emerged for a straightforward strategy to discover proteins in non-model organisms. This approach was applied in the amphipod *Gammarus fossarum*, generating a large proteome dataset and offering the opportunity to develop direct and specific analytical mass spectrometry methods. Selected reaction monitoring (SRM) is a liquid chromatography mass spectrometry based technique for the quantitation of a specific protein of interest. SRM has the ability to detect low amounts (to the picograms per milliliter range) of a targeted analyte, with tryptic peptides as multi-indicators of proteins. Absolute quantification can be performed by the addition of an isotopically internal standard. Targeted proteomics can be performed to quantify simultaneously multiple peptides per protein from the same biological sample. Multiplexing analyses by mass spectrometry is in this case straightforward and offers innovative perspectives in ecotoxicology. Using less biological material, resource investments in terms of organism and experimental maintenance are reduced and yield indicators on each biological sample. Herein, we described a highly-multiplexed MRM-based assay for the determination of proteins biomarkers in *Gammarus fossarum* an ecotoxicological model. The assay uses 71 stable isotope-labeled peptide standards for the quantitation of 40 putative biomarkers of candidate proteins related to essential physiological functions including reproductive cycle or defence mechanism. A good linearity was observed for the spiked peptides in extracted individual and, for the proof of concept, the assays precision and accuracy were determined between 0 and 20 % and between 80 and 120% respectively. The assay presented in this study is easy to use, robust and sensitive. High-throughput capabilities will be established to quantify protein biomarkers in *Gammarus fossarum*. Validation of assay was performed with protein changes assessment throughout contrasted physiological process (sex, reproductive status).

TU120

Dietary uptake of systemic fungicide contaminated plant material affects energy processing and growth of a leaf-shredder

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Leaf-shredding macroinvertebrates can be negatively affected by fungicides if they or their food are exposed to these substances via the water phase. Systemic fungicides, on the other hand, may result in an additional effect pathway; these substances penetrate plants and remain active within the vegetation, rendering possible the transport of contaminated plant parts into aquatic systems during leaf fall or storm events. In a previous study, we showed that the shredder *Gammarus fossarum* preferred microbially conditioned leaves treated with systemic fungicides over control leaves. This may be related to a higher production of carbohydrates but lower investments in self-defense of plants and consequently in the development of a microbial community more palatable for *Gammarus*. Therefore, we tested if this putatively higher quality of leaf material treated with systemic fungicides would translate into effects on energy processing and physiology when such leaves are fed over the long-term. We irrigated *Alnus glutinosa* trees (n=3) with fungicide-free water, a mixture of four systemic fungicides at recommended field rates (azoxystrobin 200 g/ha, cyprodinil 400 g/ha, quinoxyfen 100 g/ha, and tebuconazole 200 g/ha; FR), and at rates 10-times above the field levels (FRx10). The fungicides were applied twice, with a six-week interval. Leaves were picked from the trees six weeks after the last application and were microbially conditioned for 12 days before fed to *G. fossarum* in a 24 days lasting bioassay with a food and medium renewal every 8 days. Gammarids' leaf consumption, feces production and final body mass were measured. While no significant differences were found for feces production, gammarids fed fungicide-treated leaves showed a higher leaf consumption (FR by ~30%; FRx10 by ~12%), while this difference was only significant for FR. Also final body mass tended to be higher in the fungicide treatments (FR by ~5%; FRx10 by ~12%) but was significant only for FRx10. Our data suggest that systemic fungicides can affect shredders' energy processing and physiology if applied at field rates. Considering the usually much higher frequency of fungicide applications (up to 20 per season) than used during this study, further experimentation is recommended to test the effects of more realistic application patterns and their potential consequences for ecosystem structure and function.

TU121

Are waterborne and diet-related effects of fungicides transferable among leaf-shredders?

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Leaf litter breakdown is of prime importance for the energy budgets of detritus-based stream ecosystems, while an instrumental role in this process is attributed to macroinvertebrate leaf-shredders. Fungicides can, however, affect shredders via both waterborne exposure and their diet as demonstrated for the crustacean *Gammarus fossarum*. Effects mediated via the latter pathway were attributed to the adsorption of fungicides to leaf material and subsequent dietary exposure as well as changes in leaf-associated fungal communities, which are crucial for leaves' food quality for shredders. To test if effect patterns and threshold concentrations are transferable to shredder species, the present study investigated the effects of a mixture of five current-use organic fungicides (MOF) and copper (Cu) on larvae of the caddisfly *Chaetopteryx villosa*. We assessed the fungicides' effects on *C. villosa*'s feeding activity (waterborne path) as well as food choice when offered leaf material microbially colonized in the presence or absence of fungicides (diet-related path). *C. villosa* was less sensitive towards waterborne toxicity than *G. fossarum* with EC20-values being higher by a factor of three and six for MOF and Cu, respectively. In contrast, for food-choice, significant preferences where detected for *C. villosa* at exactly the same concentrations as for *G. fossarum*: a significant preference for unexposed over MOF-exposed leaves was observed at 600 and 3000 µg/L, which is probably explained by a repellent effect. In contrast, the larvae preferred leaves exposed to 250 and 500 µg Cu/L over unexposed leaves, which is most likely explained by Cu-induced shifts in the leaf-associated fungal community. Waterborne toxicity thus appears to be of comparably little relevance for the assessed caddisfly since its low sensitivity (compared to *Gammarus*) results in effect threshold concentrations much higher than field levels. In contrast, diet-related effects seem highly transferable among shredder species. Assuming also a similar relationship between effect threshold concentrations for food-choice reactions and physiological fitness effects due to long-term feeding on fungicide-affected leaf material (factor of ~10) as observed for *Gammarus*, effects via this pathway may also be detected at field-relevant concentrations for *C. villosa*. Currently, we experimentally test this assumption, while affirmative results would further support the importance of this pathway for shredders.

TU122

Corophium volutator: a model marine regulatory test species for chronic sediment testing.

M. Fox, Cefas / Environment and Animal Health

The amphipod, *Corophium volutator* is a model test species for marine and estuarine sediment toxicity testing. They are an ecologically important species to the estuarine habitat involved is sediment bioturbation and as a food source to a number of birds and fish including the Redshank, Dunlin and Flounder. *C. volutator* are already being used as a test species in the 10 day sediment reworker ecotoxicity test (OSPAR PARCOM Proctocol 1995 Part A) as part of the offshore chemical notification scheme (OCNS). There is a growing need in regulation of chemicals for test guidance on chronic marine sediment testing. The American Society for Testing of Material (ASTM) have guidance documents for chronic amphipod testing using amphipods found within United States coastal waters however there is a lack of such guidance for a European equivalent species. The European Food Safety Authority (EFSA) have produced a technical guidance document to assess the safety of feed additives for the environment, this includes feed additives for aquatic animals. The guidance for feed additives in aquatic animal feeds focuses on chronic marine sediment testing and suggests using the ASTM methods. However, the ASTM methods are for North American specific test organisms, which are difficult to source in the UK and Europe and are not as environmentally relevant to European waters as native species. The poster will contain methodology and data from a series of Good Laboratory Practice (GLP) 28 day chronic tests which assess the survival and growth (wet weight and length) of *C. volutator*. The tests conducted have been submitted and accepted for use in regulatory dossiers. Further work is being conducted to assess the potential of an extended 75 day test to incorporate a reproduction endpoint.

TU123

Population Dynamics of Parhyale hawaiensis (Crustacea: Amphipoda) in laboratory culture: Comparative endpoints as a tool to measure ecotoxicological stress

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An important step to promote ecotoxicology studies is to find an appropriate model organism that can help both acute and chronic toxicities with reproductive, standardized and comparative results on individual and population levels. The objective of this work is to describe the life cycle, fitness impairment and population dynamics of a laboratory culture of *Parhyale hawaiensis* population through the construction of age-specific life tables for obtaining population rates related to reproductive success. Attending on this manner the suggestion of refinement of specific parameters such as, intrinsic growth rate (r), reproductive potential (R0) and generation time (T) obtained from a field population. Demographic parameters were calculated from the construction of an age-specific life table through the observation of a cohort derived from a *P. hawaiensis* lab culture and compared to logistic growth models built for the same species considering data derived from the construction of a time specific life table from a field population. Differences in generation length are apparent between lab and field populations. The intrinsic rate of increase (r) are higher in lab population (cohort) because they live more and attained larger size with a higher fecundity translated in a higher net reproduction rate (R0). The net reproductive rate R0, which is the average number of female offspring per female per generation, does not provide a reasonably good comparison of absolute fitness differences between field and lab populations because the average generation time T is not the same in both situations [4]. Therefore, the measure of fitness most often used, the so-called Darwinian fitness, which is merely the total number of offspring produced by an animal over its lifetime, is not applicable. In the laboratory environment, where conditions were constant and population densities were low despite rapid population growth, the intrinsic rate of increase is probably the most reasonable single measure of absolute population fitness, even though it is calculated only for females and depends on the assumption of a constant age distribution.

TU124

Parhyale hawaiensis population parameters as ecotoxicological endpoints

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Population responses to environmental stress have been evaluated for effects on intrinsic growth rate based on evidence obtained by the established association between declining growth and increased stress and environmental carrying capacity, defined as the size of the population where the population growth is zero, crucial to the perception of toxic effect in natural environments. The objectives of this study include the description of the structure and dynamics of a natural population of *P. hawaiensis* through the construction of time-specific life table in order to obtain population vital parameters to be applied at endpoints for chronic toxicity tests known as LTRE (Life Table Responds Experiment). Such demographic statistics can be used in response to exposure to toxic agents, as well as serve as basis for evaluating the welfare of cultures kept in the Laboratory of Ecotoxicology and Environmental Microbiology Dr. Abílio Lopes, Faculty of UNICAMP Technology - FT / UNICAMP. Using the data of 12 monthly time-specific life tables the parameters mean reproductive potencial (R0), generation time (T) and intrinsic growth rate (r) were calculated in order to model the logistic growth curves. The model was built considering the fecundity obtained through field data as well as lab culture. Values of R0, T and r obtained are presented as the mean values for the 12 monthly samples as 1.56 (+/-0.15), 3.75 (+/-0.35) and 0.09 (+/-0.05) respectively. The model indicates that considering field fecundity and an initial population of 10 individuals, the final population would reach carrying capacity (K) in approximately 35 generations and for lab fecundity in approximately 25 generations. The use of the intrinsic growth rate (r), the reproductive potential (R0) and the generation time (T) is recommended as more potentially effective endpoints for ecotoxicological chronic test with the suggestion that they are refined by experiments with laboratory cultures at controlled conditions. Such basic information on the test species chosen must be seen as of paramount importance if population parameters are to be effectively used as an endpoint in ecotoxicology.

TU125

Assessing estuarine quality: A cost-effective in situ assay with amphipods

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In situ assays based on feeding depression can be powerful ecotoxicological tools that can link physiological organism-level responses to population and/or community-level effects. Amphipods are traditional target species for toxicity tests due to their high sensitivity to contaminants, availability in the field and ease of handling. However, cost-effective in situ assays based on feeding depression are not yet available for amphipods that inhabit estuarine ecosystems. The aim of this work was to assess a short-term in situ assay based on postexposure feeding rates on easily quantifiable food items with an estuarine amphipod. Experiments were carried out under laboratory conditions using juvenile *Echinogammarus marinus* as the target individual. When 60 *Artemia franciscana* nauplii (as prey) were provided per individual for a period of 30 minutes in dark conditions, feeding rates could be easily quantified. As an endpoint, postexposure feeding inhibition in *E. marinus* was more sensitive to cadmium contamination than mortality. Assay calibration under field conditions demonstrated the relevance of sediment particle size in explaining individual feeding rates in uncontaminated water bodies. An evaluation of the 48-h in situ bioassay based on postexposure feeding rates indicated that it is able to discriminate between unpolluted and polluted estuarine sites. Using the harmonized protocol described here, the in situ postexposure feeding assay with *E. marinus* was found to be a potentially useful, cost-effective tool for assessing estuarine sediment and water quality.

Biomonitoring of contaminants in the marine environment: integration of biological and chemical approaches (P)

TU126

Between-year correlation between contamination in blue mussels and recruitment of cod

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We analysed two sources of data: (1) monitoring data on concentrations of eight contaminants in blue mussels from four areas sin the 1980s, and (2) annual abundance of juvenile (6 months old) Atlantic cod collected in beach seine, in the same years and in the same four areas. A statistical analysis showed that for three of the eight contaminants (HCH-A, HCB, and PCB-153), the optimal model (according to the AIC criterion) included both area (1-4) and year-specific contaminant concentration in mussels. In all cases the covariation between contaminants and recruitment was negative. However, the effect was quite variable. Among the four areas (as opposed to among years), there was no correlation between contaminant burden and abundance of cod recruits. Thus, there appears to be a link between year-to-year variation in contaminant concentrations and recruitment variations in Atlantic cod, when the general difference in recruitment among areas is been taken into account. A possible explanation for the covariation is that both mussel contaminant concentrations and cod recruitment may be affected by the same factors (e.g., climate).

TU127

Seasonal variability in biomarkers in the mussel Mytilus trossulus from the northern Baltic Sea

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With the enormous number of different substances having potentially or known hazardous properties for marine life makes it practically impossible to assess their risks by chemical measurements only. Furthermore, chemical measurements do not account for long-term sublethal effects or mixture toxicity. To complement chemical measurements in monitoring and assessment of hazardous substances, the use of biomarkers is widely considered as a valid choice. In using biomarkers it is of crucial importance to understand natural variability not related to anthropogenic contaminants; this includes seasonal variability caused by abiotic and biotic factors. Many hazardous substances are known to cause pressure to the oxidative defence system of organisms, measured as responses in antioxidant molecules or biological damage, leading to the state of oxidative stress. However, natural variability in factors such as temperature, nutrition and reproductive status can also cause oxidative stress, often by increasing the metabolism of organisms and, hence, production of reactive oxygen species. In the current work, seasonal variability in selected biomarkers was studied in the mussel *Mytilus trossulus* from the northern Baltic Sea by monthly samplings. The biomarkers measured were lipid peroxidation, superoxide dismutase activity, protein carbonylation and lysosomal membrane stability. In addition, the condition index (CI) of the mussels was determined. All the studied biomarkers showed significant seasonal variability with the most stressful period occurring in May. In springtime, multiple factors are likely to contribute to the observed elevation in oxidative stress in mussels, including the sudden marked increase in available nutrition after the intensive spring phytoplankton bloom, and preparation for spawning. Also, meltwater runoff in coastal areas can temporarily contribute to environmental factors. Based on this study, biomarker analyses for monitoring and assessment of hazardous substances using mussels should in this sea area be avoided during the spring and early summer period. Furthermore, routine examination of the CI is recommended as this provides a good basis for assessing the general health status of the mussels that is often linked with the background levels of many biomarkers.

TU128

Assessment of the state of 2 populations of pearl oyster Pteria sterna

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In this study, an assessment of the state of *Pteria sterna* populations located at 2 sites was performed: a pristine area (Gaviota Island BCS) and an area with anthropogenic influence (La Marina BCS) using 3 biomarkers: Degree of lipid peroxidation, acetylcholinesterase activity and genetic damage (micronuclei). In December 2011, July 2012 and December 2012. fifteen organisms of each site was taken and the evaluation of biomarkers was performed in their gill tissue (lipid peroxidation and genetic damage) and foot (Ache). The results indicated that the most adverse times to the organisms that are found in La Marina was December 2011 and Dicember 2012, at this time important contributions of xenobiotics to this site were detected due to increased rainfall. And bivalves that inhabit on Gaviota Island were December 2011 and July 2012, dates which coincide with increases in water temperature caused by "El Niño" phenomenon. Biomarkers were a useful tool to detect the influence of xenobiotics in juvenile *Pteria sterna*. The degree of lipid peroxidation and evaluation of micronuclei were the most sensitive biomarkers to detect "stressful situations" that occurred in the Marina during the stormwater contributions.

TU129

Application of a battery of bioassays for assessing the effects of WWTP effluent on the aquatic biota

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Since chemical and biological data is considered important for assessing the quality of municipal wastewater treatment plant (WWTP) effluent and its environmental risk, the present study investigates the effects of WWTP influent and effluent on a battery of marine organisms such as the brine shrimp *Artemia franciscana* (formerly *Artemia salina*), the sea bream *Sparus aurata,* and the green algae *Dunaliella tertiolecta,* while primary culture of hemocytes of the marine bivalve mollusk *Mytilus galloprovincialis* was also used for investigating the cytotoxic and oxidative effects of samples. Chemical analysis, primarily performed in samples, showed that WWTP processes efficiently reduce the levels of each parameter tested in effluent. On the other hand, the exposure of the green algae *Dunaliella tertiolecta* to different concentrations of each sample (6.25-50% v/v), showed a significant dose-dependent decrease of WWTP influent-treated algae growth rate, after 24h, compared to that observed in WWTP effluent-treated algae, a fact that attenuated over time (96h). However, a significant depletion of algal growth rate was observed in cells treated with concentrations of WWTP influent higher than 25% v/v. Similarly, a slight attenuation of WWTP effluent toxic effects was observed in the brine shrimp *Artemia salina*, compared to those occurred in case of WWTP influent (24hLC_{50} values of 90.63% and 86.58 v/v respectively). Moreover, *Sparus aurata* larvae incubated with different amounts of WWTP influent and/or effluent (0.001-10% v/v) for 6 days, showed a time-dependent increase of larvae mortality rates. However, among live larvae, significantly elevated developmental abnormalities/deformities were obtained in WWTP influent-treated larvae, at least in case of larvae treated with 10% v/v of influent. Regarding WWTP effluent-mediated cytotoxic and oxidative potency, a significant increase of cell death as well as increased levels of lipid peroxidation were observed in WWTP influent-treated hemocytes of mussels, compared to those occurring in WWTP effluent-treated cells. In conclusion, the results of the present study showed that even if WWTP processes could efficiently remove organic compounds and pathogenic microbes from municipal influent, WWTP effluent disposal could pose a risk for the aquatic environment. The extent of WWTP risk seems to be species-specific, depending mainly on species' vulnerability and sensitivity.

TU130

Imposex in the dogwhelk (Nucella lapillus): twenty two years monitoring around England and Wales

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Six imposex surveys in the dogwhelk (*Nucella lapillus*) have been conducted over the past two decades to assess legislation effectiveness controlling the use of TBT by the maritime shipping industry. This study firstly analysed the results of the 2014 survey and secondly carried out a trend assessment of the same 88 sampled sites between 1997 and 2014 of which 65 showed statistically significant reductions. To highlight the magnitude of change the VDS of the same 56 sites sampled in 1997 and 2010 showed that the VDSI reduced statistically significantly from 2.89 and 0.42 respectively. These data confirm that the legislation enacted, latterly through the IMO during 2003 to 2008, has been effective in progressively reducing the impact of TBT in the marine environment. Nevertheless, the 2014 results show that two of 18 sampled sites (Gurnard Bay and St. Mawes) are still above the EAC derived for TBT (VDSI≤2).

TU131

Caging approach comparison of the mussel Mytilus sp and of the sponge Hymeniacidon perlevis in assessing biological effects of harbour seawaters subjected to metallic and organic contaminants

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Since these last years, different approaches were settled to assess seawater quality in the aim to improve the good chemical status of coastal waters demanded by EU-WFD. One approach is to provide biological model (bioindicator) as environmental sentinel of harmful chemical introduction. The marine model organism which is often retained as integrative matrix of contaminants is the mussel (*Mytilus edulis*), especially within the French networks set up since 1970's years. This species presents criteria retained for bioindicators such as abundance, sedentary, tolerance to physico-chemical variations, and can integrate chronic contaminations of "water masses" over a large period. Moreover, this organism presents some limits as integrative model linked to its reproductive cycle which imposes specific period of study. Since these last years, the research of new bioindicators such as sponges has been developed. The sponges are sessile species without organs (in opposition to bivalve molluscs) whose morphology is adapted to maximize the efficiency of water flow. After a specific study to the identification and distribution of the sponges populations present along the coasts of Normandy, one species (*Hymeniacidon perlevis*) showed promising results in accumulation of different chemical compounds such as metallic and organic contaminants (PCB and HAP). To demonstrate the interest of using this species as bioindicator, natural and local samples of *H. perlevis* and *M. edulis* were transferred in same time by caging in different sites of one harbor in Normandy. Organisms were then exposed to different anthropic sources of contamination in order to compare their accumulation performances. In parallel, tests were performed on this sponge set up in mesocosms to assess copper bioconcentration factor. The aim was to evaluate the in-situ harbor seawater concentration of this element strongly accumulated in sponge tissues. Results will be presented in this work and discussed in the perspectives of the Water Framework Directive.

TU132

Biological-chemical parameters' coupling for the monitoring of trace metal contaminants in food webs: the case study of a land-to-sea continuum in the North-East Atlantic

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Biomonitoring of chemical contaminants is positioned at the biological-chemical interface, requiring skills and knowledge in various domains – analytical chemistry, biogeochemistry, biochemistry, ecology and physiology of organisms – in order to be useful to fulfil its role: describe levels of chemicals, interpret them, and assess their potential effects. Biomonitoring can therefore provide a global picture of contaminants in systems, even if general strategies must be amended to fit site-specific constraints such as local physico-chemical forcing variables, occurrence of monitored species, etc. Here, we propose to combine both chemical (metal concentrations, carbon (C) and nitrogen (N) stable isotopes measured in various species), and biological parameters (metallothionein (MT) concentrations, data on the ecology of organisms) to describe contaminants in food webs, taking the Loire estuary as a case study. Thus, ten species (including 7 fish, 2 crustacean and 1 bivalve species) representative of the food web were analysed for these parameters. C and N isotope results traced the different sources organic matter sustaining the different species, assessed their habitat and trophic level, and consequently were crucial in interpreting metal burdens. MT concentrations and metal distribution between soluble and insoluble fractions also revealed differential metal regulation and/or detoxification capacities among the species. For instance, among fish, the microphytobenthos-grazer (then low trophic level) mullet *Liza ramada* presented the highest metal concentrations probably due to sediment exposition, but exhibited an efficient mechanism of regulation and/or detoxification (high MT levels and metal insolubilisation). This integrative approach adds to quantitative monitoring of environmental contaminants because it provides a view of the effective bioavailability of chemicals, and of their transfer and bioaccumulation in food webs – a major component of ecosystems. This study therefore proposes that the combined use of such tools has considerable interest in monitoring programmes that aim at describing the temporal and spatial contaminant variability in ecosystems. Finally, in the perspective of the European Marine Strategy Framework Directive (MSFD) implementation, it represents a concrete example of biological-chemical parameters' coupling to document both MSDF descriptors D4 (Food-web) and D8 (Pollution), and to more broadly monitor contaminants in food webs.

TU133

Genotoxicity assessment in the spermatozoa of the common prawn Palaemon serratus using the alkaline Comet assay

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Sperm quality is essential for reproduction success. Thus there is an increasing concern to understand how the xenobiotic exposures affect the structure of spermatozoa. The measure of DNA integrity appears as an interesting and relevant marker to assess the sperm quality. Nowadays, majority of ecotoxicology studies was relatively narrow to molluscs and fishes despite the interest to investigate the different phyla. Crustaceans and particularly the marine and estuarine species are still rarely studied despite their ecological importance. The purpose of this study was to develop and optimize the alkaline comet assay in marine and estuarine *Palaemonidae* prawns to assess sperm quality. In first times, an optimization of procedures including the collection of spermatozoa, the maintenance in appropriate media as well as different steps (*i.e.* lysis, denaturation) of the comet assay was performed in *Palaemon serratus*. Then the sensitivity of cellular model was assessed using a variety of well-known genotoxicants with diverse modes of toxic action (*i.e.* UV, H_2O_2, MMS). Results attested the reliability and the sensitivity of the procedure developed. In the second times, DNA integrity of spermatozoa was measured in differentially impacted *Palaemon sp.* populations along the Bay and the Estuary of Seine (*i.e.* the coastal species *Palaemon serratus,* and the estuarine species *Palaemon longirostris*) during the year 2015. The first results suggest important DNA damages in populations from the Seine estuary and a lower effect in populations away from the estuary. In conclusion, this work showed the pertinence and the potential of the alkaline comet assay on *Palaemonidae* spermatozoa in the monitoring of environmental genotoxicity.

TU134

Immune capability of humpback whale cell lines and application in immunotoxicity

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Humpback whales have an elevated sensitivity to accumulate persistent organic pollutants (POPs) and chemical pollution may impact animals' health. Measuring the immunotoxicological impact of POPs on wild populations of humpback whales is challenging, however, toxic effects in humpback whales are still poorly understood. In this study we use commercial multiplexed ELISA microarrays as a screening tool to measure and quantify relevant proteins. Gene ontology and ingenuity pathway analysis are applied to identify protein targets involved in immune responses and disease processes. In the used approach over 160 proteins were detected and revealed a big overlap between humpback whale derived cell lines and dermal humpback whale skin biopsies. The cell lines were capable of immune stimulation. Incubation with RAW264.7 conditioned media resulted in concentration-dependent expression of IL-6, TNFα and I-Tac. Immune screening and GO enrichment analysis exhibited overrepresented annotations associated with immune system processes, response to external stimuli and chemotaxis. A multiple stressor approach is applied to analyse the impact of POPs on the immune capability of humpback whale cells. The used microarray is a powerful tool for screening and identification of potential biomarker protein targets associated with immune responses in humpback whale cell lines and dermal biopsies. Thus, we provide a minimally invasive method for the study of the whales' sensitivity and cellular response to chemicals and other environmental stressors.

TU135

Multiple stress effects on marine planktonic microalgae

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In the present context of global warming, more knowledge on the combined effects induced by temperature changes and mixtures of relevant environmental contaminants is urgently needed. Therefore, the main objective of this study was to investigate the effects of temperature increase (20ºC to 25ºC) on the toxicity of one metal (cadmium) and one polycyclic aromatic hydrocarbon (benz(a)anthracene), alone and in mixture, to the marine planktonic algae *Tetraselmis chuii*. Microalgae cultures were exposed for 96h to different concentrations (0.04 - 20 mgL^{-1}) of the two substances individually and in binary mixtures, at 20 and 25 °C. The effect criterion was the inhibition of culture growth. The increase of temperature by 5 °C significantly increased the toxicity of both contaminants. Toxicological interactions among the stressors were found. The findings of this study highlight the need of more research on the effects of temperature on the toxicity of chemical mixtures.

TU136

Eco-toxicological assessment of lithium adsorbents and long-term monitoring of marine microorganism at lithium-recovery field site

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The adsorbents which made by lithium manganese oxide for recovery of mineral resources from seawater were developed by KIGAM (Korea Institute of Geoscience and Mineral Resources) and the effectiveness of lithium recovery was verified. When the field application of large amount of adsorbents in ambient seawater (Okgye Harbor, Gangneung, Korea), it should be consider the impact on marine environments by lithium and manganese. In this study, long-term and periodic monitoring has being carried out to detect changes of marine general factors(i.e. chlorophyll, standing crops of phytoplankton, and concentrations of trace metals) at lithium recovery pilot plant and eco-toxicological effects on marine environment by seawater exposure of lithium adsorbents. First, abundances and species of phytoplankton have been evaluated through monthly interval sampling from February 2013 to May 2015 since phytoplankton is important indicator of primary productivity of marine. Abundance and species diversity of phytoplankton went up to summer from winter during whole periods. Eco-toxicological assessment of lithium adsorbents was performed with Microtox using bioluminescence bacteria *Vibrio fischeri*. The lithium adsorbents was soaked in sterilized seawater and aeration for 1, 3, 5, 7, 10 and 14-day intervals under controlled temperature. As the results, toxicities of adsorbents were found in more than 10 days exposure and maximum EC_{50} concentrations were 61.4%. At this time, concentration of eluted metals such as As, Cd, Cr, Cu, Zn, As, Sn, and Pb were also higher. The study is in progress about eco-toxicological assessment as maximum allowable concentration with species sensitivity distribution (SSD) of monitoring factors. **Acknowledgement** This research was supported by the national research project titled "The Development of Technology for Extraction of Resources Dissolved in Seawater" of the Korea Institute of Geoscience and Mineral Resources (KIGAM) funded by the Ministry of Oceans and Fisheries (Project No. PKF061).

TU137

Toxicity of herbicides used to control the invasive weed Spartina anglica to four invertebrate species inhabiting an Australian saltmarsh

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Spartina anglica is recognised as an invasive weed in Australia and as a threat to local flora and fauna. Control efforts include the use of the herbicide Fusilade Forte with the adjuvant Hasten . Anderson Inlet, Victoria, Australia is subject to routine spraying of herbicides for *Spartina* control and little is known of the effects of these chemicals on local invertebrate species. Four species commonly found in Anderson Inlet, *Lumbriculus variegatus, Aglaophamus australiensis, Tatea rufilabris,* and *Allorchestes compressa,* were tested in the laboratory with Fusilade Forte and Hasten alone and as a mixture, using 96 hr acute toxicity tests to estimate median lethal concentrations (LC_{50}). *A. compressa* was the most sensitive species in all tests, and *T. rufilabris* the most tolerant.The herbicide mixture was more toxic to the three marine species, *A. australiensis, T. rufilabris, and A. compressa* than Fusilade Forte alone, with toxicity increasing by 146%, 129%, and 181% respectively. However the mixture was less toxic than Fusilade Forte alone by at least 25% to the freshwater species, *L. variegatus*,. Sub-lethal tests on *T. rufilabris* also revealed a higher toxicity of the mixture compared with the toxicity of Fusilade Forte. The laboratory results indicate that using Fusilade Forte with Hasten is more toxic to non-target marine invertebrates than using Fusilade Forte alone. However, consideration of dilution scenarios in the field suggests that rapid degradation and dilution from the incoming tide would reduce the concentrations to well below the LC50 values measured here, resulting in low risk of acute toxicity to aquatic invertebrates from these herbicides in field sites. Monitoring of field invertebrate communities for a period of one to six months after spraying of herbicides as Fusilade Forte alone or as a mixture, yielded little evidence of detrimental effects.

TU138

Organohalogen compounds in fish biota of Guanabara bay, Rio de Janeiro, Brazil

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OCPs have been reported as an environmental concern issue, since last decades. Nevertheless, their global threats are still difficult to access due to most researches are focused on selected groups of contaminants while other groups remained unstudied. Persistent organic pollutants are regulated under the Stockholm convention since 2001 and constitute a diverse group of organic substances, which are toxic, persistent, bioaccumulative and tend to long-range transport. Urbanization and industrialization severely contribute to contaminated hotspots and worldwide distribution of organohalogen pollutants. The most anthropogenically disturbed area along the brazilian shoreline is Guanabara bay, situated in Rio de Janeiro state. This estuary is bordered by 12000 industries and four cities, with a total population of about 11 million people. Despite the anthropogenic pressure, Guanabara Bay supplies food and breeding grounds for several wildlife, which makes it an important fishing point to the local market and

population. Focusing on expand the knowledge about organohalogen compounds, we tried to determine some target compounds classified as POPs and non-target compounds, in three fish species with high level consumption, by gas chromatography (GC) coupled to electron capture negative ionization (ECNI) with a quadrupole mass spectrometer (MS). The profile contamination of POPs was mainly composed by polychlorinated biphenyls (PBCs) followed by legacy organochlorine pesticides (OCPs) and PBDEs both natural and anthropogenic compounds, according to the literature. Moreover, the presence of some organohalogen compounds less commonly analyzed as the heptachlorobipyrrole (Q1) and pentachloroanisole (PCA) were found in all samples. Regarding these non-target compounds, Q1 has been previously reported in Guanabara Bay by Rosenfelder *et al*, 2012, in a study which they also document the presence of a new DDT metabolite in the local biota. Pentachloroanisole is a common metabolite of pentachlorophenol (PCP) in fish, although it is not well documented. This product is only used in Brazil in wood protection since 1998, before that it was used as fungicide, algaecide and insecticide. As depicted by our results, legacy and emerging POPs can be easily found in the high polluted Guanabara Bay. This may represent a health risk hazard to fishermen and their families. We suggest that more research ought to be done in order to clarify this issue in the near future.

TU139
Acute and chronic toxicity of chlorine to selected marine species of the Arabian Gulf waters
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Chlorine is extensively used as a powerful oxidizing agent in the countries surrounding the Arabian Gulf for water treatments and biofouling control. The usage has been increasing significantly as demand for water grows considerably both in industry and domestic use. This is due to the fact that it is a well-tested technology, has had a history of long-term worldwide industrial use and is of acceptable cost. In seawater, chlorine produces a mixture of hypochlorous acid and hypochlorite ion. These rapidly react with the bromide ion to form a mixture of hypobromous acid and hypobromite ion. The acute oxidants formed by chlorination are therefore short lived and are not persistent in seawater, but can be quite toxic. Further complicating the environmental concern of chlorination is the production of numerous, and more persistent, compounds formed by complex reactions between chlorine/bromine and the organic constituents of seawater, collectively described as chlorination by-products (CBPs). Many CBPs are persistent and may be toxic to marine organisms subjected to long-term exposures. In order to evaluate the risks of chlorine exposure to Arabian Gulf marine organisms, the aims of this study were to 1) develop protocols for acute and chronic toxicity tests involving native species at different trophic levels, 2) correlate sensitivity to other marine organisms used as indicator species in risk assessments, 3) draw conclusion from the results and explore ways that this could be used for informing environmental management activities. Species were collected from different locations around the coastal areas of Qatar. They were then cultured in the laboratory using conditions of the Arabian Gulf. Species used in the tests include phytoplankton (*Synechococcus sp*.), zooplankton *(Euterpina acutifrons),* pearl oysters *(Pinctada radiata),* sea urchins *(Diadema setosum)* and killifish embryos (*Aphanius dispar)*. Different concentrations of chlorine were either administered as a continuous flow through or via daily renewal. Sensitivity varied across the trophic levels for each species and was found to be in line with other test organisms that are used in established toxicity procedures. All five species used were found to be useful for certain types of toxicity testing. For example, *Pinctada radiata* and*Diadema setosum* were excellent in testing for successful fertilization and early development, while local *Aphanius dispar* embryos were useful for following sub-lethal effects such as malformation and hatchability. Embryos of oysters and urchins were found to be the most sensitive to chlorine. Effect of chlorine was only observed near or after hatching has occurred. Continuous exposure showed more effective than the semi-renewal method. The purpose of this study was to increase the knowledge of chlorine chemistry and toxicity especially the sensitivity towards the Arabian Gulf species. Results obtained reveal that the sensitivities of all five species used in this study are in line with species used internationally in similar tests. Expanding tests with species used in this study to evaluate their sensitivity towards chlorine by-products will further increase our understanding of their chemistry and environmental risk in the Arabian Gulf and will provide a science-based tools for making management decisions. \n <br clear="ALL" />

TU140
The IPOC project : Interactions between POllution and Climate changes, Development of improved monitoring strategy
M. AUFFRET, IUEM / LEMAR UMR; M. FOURNIER, Université du Québec À Rimouski / ISMERUQAR
Anthropogenic pressure (habitat destruction, chemical contamination, eutrophication) is recognized to induce deleterious effects on aquatic ecosystems, especially coastal waters whose production is of major importance for humans. Ongoing climate changes are expected to generate in a next future unexperienced, stressful environmental conditions which will probably deeply affect biota in targeted habitat as rivers and estuaries. These natural systems are linked as a continuum through water transfers. Coastal, intertidal habitats are naturally highly variable, both spatially and temporally. Such environmental conditions make them highly exposed to synergistic effects of pollution and temperature stress. At the basis of the trophic chain, benthic invertebrates, especially filter-feeders, control ecosystem productivity by locally producing a huge amount of biomass and should now be considered as key-stone populations at risk. The project IPOC (2013-2016) is a joined, international project borne by French and Canadian laboratories and companies. This consortium will open research into an exceptionally broad range of habitats, from Artic to Austral regions, for observations in natural populations of mussels (*Dressena spp* and *Mytilus spp*). IPOC scientific rationale relies on a better understanding of the sensitivity of these sentinel species in the continuum freshwater-to-coastal areas and thus their vulnerability to environmental stress. The approach combining observation and experimentation will allow to: 1. Assess the diagnostic capacity of a selection of molecular, cellular and physiological biomarkers for assessing biological effects of the combined action of major anthropogenic stressors in aquatic environments 3. Provide the scientific community and environmental managers with an improved "tool-kit", based on original, up-to-date scientific knowledge, for long-term monitoring of aquatic environments, according to recommendations from international organizations 2. Foresee the capacity of aquatic populations at risk to cope with environments altered by the global change and further, possible alterations of local ecosystem services Keywords: Aquatic ecotoxicology, anthropogenic pressure, bivalve mollusks, biomonitoring *The research project IPOC has been funded by the ANR and NSERC Agencies (joined project France-Canada) for the 2013-2016 period.*

TU141
QUASIMEME development exercises to improve world-wide reliability of data in the marine environment
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An integrated biological-chemical monitoring and assessment of chemical pollution in the marine environment is not only a matter of using chemical analytical methods, biological effects methods such as biomarkers and a modelling tool for interpretation. It needs reliable data and common approaches. Results from different countries including their laboratories are often involved in the necessary investigations. Results from these laboratories should be comparable and used in one single assessment model. Standardization and proficiency testing is not always a solution to improve the comparability of laboratories. Especially if methods are still in development, standardization is not yet an option. Exchange of information by involved scientists and technicians as promoted in the QUASIMEME development exercises has shown to be an effective tool to improve results. QUASIMEME stands for Quality Assurance of Information for Marine Environmental Monitoring. At the heart of the programme is a holistic learn-by-doing spiral. The laboratory performance studies provide the basis of external quality assurance for institutes that make regular measurements in the marine environment. For routine parameters a performance study can be organized on regular basis. For new parameters a more fit to purpose approach is necessary as used in the QUASIMEME development exercises. These improvement programmes may be initiated through a workshop and with a series of Development Exercises to provide detailed tuition, information and test materials tailored to the specific needs of the problem. The workshops to exchange information and experiences are essential. Successful exercises has been organized for perfluorinated compounds, chlorinated paraffins, marine biotoxins, organotin compounds, chlorophyll and passive sampling using seawater, sediments and aquatic organisms. Because a large number of marine laboratories are already member of QUASIMEME, it will not be too difficult to extend the approach to other and new quality parameters in the marine environment.

Development and application of oxidative stress biomarkers and models in ecotoxicology and environmental monitoring (P)

TU142
Integrated chemical and toxicological profiling of surface water samples from Swedish agricultural areas
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Pesticides applied on agricultural land can contaminate ground- and surface water and adversely affect ecosystems and drinking water quality. As part of a Swedish program on environmental monitoring of pesticides, we have collected 161 surface water samples from six small catchment areas representing four large

agricultural regions and two rivers, during the years of 2013-2014. The water samples have been analyzed for residues of 131 pesticides, using GC-MS and LC-MS/MS. Of the analyzed pesticides, 87 were detected in the water samples. The water samples were also used for toxicological profiling *in vitro*, using cellbased bioassays. Oxidative stress response is a sensitive indicator of toxicity, responding to a wide variety of chemicals. We investigated the oxidative stress response exerted by the water samples, by measuring the Nrf2 activity. Furthermore, we measured the aryl hydrocarbon receptor (AhR) activity, indicating induction of metabolism of xenobiotics and cytotoxicity by MTS test. The water samples from the pesticide monitoring program were extracted, concentrated and then analyzed by *in vitro* toxicity testing. No cytotoxicity was observed. A large number of the water samples exerted significant induction of the Nrf2 activity and the AhR activity. The effect was particularly strong in the AhR assay, where 123 of the water samples activated the receptor >2 fold compared to the control. Oxidative stress (Nrf2 activity >1.5 fold compared to control) was exerted by 28 of the water samples. The results did not indicate a correlation between AhR and Nrf2 activities. A correlation was found between Nrf2 activity and season. By stepwise regression a statistically significant positive correlation was found between Nrf2 activity and concentration of five pesticides: diuron, lindane, metazachlor, rimsulfuron and terbuthylazine. These effects remained when area and season were included in the model. In conclusion, we found that a large number of the water samples contained substances that exert oxidative stress and activates AhR *in vitro*. No correlation between the two parameters was found but a positive correlation between Nrf2 and some of the pesticides monitored in the program. The study demonstrates that these bioassays are valuable tools to monitor water quality and evaluate environmental effects of pesticide residues.

TU143

Nrf2 as an oxidative stress biomarker of drinking water from source to tap - association with AhR activity and chemical pollutants

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Drinking water contains a complex mixture of known and unknown chemicals, where the individual compounds may be present in concentrations too low to cause adverse effects, but added together may result in combination effects. Oxidative stress response is a sensitive indicator of toxicity, responding to a wide variety of chemicals. The objective of this study was to evaluate the influence of different steps of water treatment on oxidative stress response by measuring Nrf2 activity. In addition chemical pollutants, cytotoxicity and AhR activity were analyzed in the water samples. Water was sampled from five sites before the intake (including up- and downstream a waste water treatment plant (WWTP), after a sand filter, after a granulated active carbon filter, at the outlet of the drinking water treatment plant (DWTP), and from a nanofilter pilot plant. The samples were concentrated by solid phase extraction with two different adsorbents, HLB and ENV. Chemical analysis was performed by LC/TOF-MS for 124 target chemicals, mainly medical drugs, pesticides and household chemicals. In almost all water samples, with a final concentration factor of 50, Nrf2 and AhR activities were detected. Raw water samples had in general higher activities than samples from inside or taken after the DWTP. No evident decrease in activity from a specific treatment step in the DWTP was detected, except in the nanofilter pilot plant, where activities of Nrf2 and AhR decreased after a filter of old granulated active carbon. An association between Nrf2 and AhR activities was found in many of the water samples. Preliminary results did not indicate any genotoxic activity in the water samples, as measured by Comet assay. No cytotoxic effects were revealed in the water samples. The total concentration of target chemicals followed another pattern and decreased considerably from downstream the WWTP to the inlet of DWTP. No Nrf2 or AhR activity was detected in a sample spiked with all the target chemicals, indicating that the activities in the water samples were not caused by the target chemicals and that other, yet not identified compounds, are responsible for the observed activities. The study demonstrates that these bioassays are valuable tools to monitor water quality and evaluate effects of water treatment.

TU144

Fungicide prochloraz induces oxidative stress and DNA damage in vitro

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Prochloraz is widely used in horticulture and agriculture, *e.g.* as a post-harvest anti-mold treatment. Prochloraz is a known endocrine disruptor causing developmental toxicity with multiple mechanisms of action. However, data are scarce concerning other toxic effects. Since oxidative stress response, with formation of reactive oxygen species (ROS), is a common mechanism for different toxic endpoints, e.g. genotoxicity, the aim of this study was to investigate if prochloraz can induce oxidative stress and/or DNA damage in human cells. We used a cell culture based in vitro model to study oxidative stress response by prochloraz, as measured by the activity of the nuclear factor erythroid 2-related factor 2 (Nrf2), a key molecule in oxidative defense mechanisms. We found that prochloraz is able to induce oxidative stress in cultured human adrenocortical H295R and hepatoma HepG2 cells at non-toxic concentrations. Further, we used Comet assay to investigate the DNA damaging potential of prochloraz, and found that prochloraz at non-toxic concentrations is able to induce DNA damage in HepG2 cells. These are novel findings, contradicting previous studies in the field of prochloraz and genotoxicity. This study reports a new mechanism by which prochloraz may exert toxicity. Our findings suggest that prochloraz might have genotoxic properties.

TU145

BEHAVIORAL, OXIDATIVE STRESS AND GENOTOXICITY EFFECTS IN THE POLYCHAETE NEREIS DIVERSICOLOR TO THE CYTOTOXIC DRUG CISPLATIN

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\n Anticancer drugs inhibit the process of cell replication and transcription through interaction with DNA, inducing potential mutagenic, cytotoxic, genotoxic and carcinogenic effects. Concerns about their fate and long-term effects in aquatic invertebrates are arising, due to the continue discharge in waterbodies by municipal and hospital effluents. The aquatic sediment represents an important reservoir for those compounds that can pose a risk to the benthic biota. Guidelines for sediment quality assessment does not include pharmaceuticals as contaminants of concern, and little attention has been paid to the potent effects of cytotoxic drugs to non-target organisms. Cisplatin (cis-diammine-dichloroplatinum II) is an alkylating agent globally administered, which environmental levels range from ng $Pt.L^{-1}$ to μg $Pt.L^{-1.}$ The present study aimed to assess the potential effects of the anticancer drug cisplatin to the benthic ragworm *Nereis diversicolor*, at environmental relevant concentration and worst-case scenarios addressed in waterbodies. Polychaetes were exposed to cisplatin at 0.1, 10 and 100 ng $Pt.L^{-1}$, in systems containing sediment and water (1:4), over 14 days. The multibiomarker approach involved a behavioural assessment and biochemical analysis, including neurotoxicity (AChE), antioxidant enzymes (SOD, CAT, GPXs, GST), and oxidative damage (LPO). Genotoxicity was performed through comet assay. Results showed neurotoxicity in animals exposed to the 100 ng $Pt.L^{-1}$ concentration, by the significant inhibition of AChE ($p< 0.05$). Likewise, the activity of antioxidant system was significantly impaired by depletion of SOD, CAT and GST levels, and overexpression of GPx, at the highest concentration of the drug, which lead to high levels of lipid peroxidation products. DNA damage was not detected in polychaetes as a consequence of intra and interstrand cross-links in DNA, associated to the cisplatin mode of action. The multibiomarker approach confirmed the sensitivity of the polychaete *N. diversicolor* to a potent pharmaceutical of cytotoxic activity, addressing that low concentrations combined with chronic exposure may represent a hazard for representative benthic species of coastal waters.

TU146

Towards an early molecular response in periphytic diatoms to reveal impacts of various contaminants: quantitative gene expression

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This poster aims to highlight the potentialities of the gene expression tools in diatoms to reveal the different impacts of various contaminants which may be found in the natural aquatic systems. Through three independent exposure experiments of a periphytic diatom species to pesticides (diuron), to metal (cadmium) and to metallic nanoparticles (AuNP), the use of gene expression has shown its efficiency to point their cellular impacts and the metabolic functions affected. Diatoms are unicellular algae responsible for a large proportion of vegetal primary production in upstream watercourses and have therefore an essential role at the basis of the freshwater trophic web. They are excellent ecological indicators at the species level, sensitive to a number of environmental variables (light, temperature, inorganic nutrients) and respond to many types of pollution (eutrophication, organic and metallic pollution). Thus laboratory experiments have been performed to expose different diatoms species: *Eolimna minima*, *Achnanthidium minutissimum*, *Nitzschia palea*, *Gomphonema parvulum and Planothidium lanceolatum* cultivated in lab to various contaminants during 48 hours to 2 weeks. A quantitative global criterion has been estimated (total density) to follow their growth in the experimental conditions in parallel to molecular biology analyses. The expression level of target genes involved in the mitochondrial metabolism (*cox1*, *nd5*, 12S), response to oxidative stress (*sod* Cu/Zn, *sod* Mn, *cat*), detoxification (phytochelatin synthase) and photosynthetic processes (*psaA, d1*), has been determined by real time quantitative PCR. Genetic responses evidenced the impact of pesticides, trace metal and nanoparticules on the mitochondrial metabolism, oxidative stress response and the chloroplast photosystem II. Moreover, the analysis of gene expression was evidenced to constitute an early biomarker of alert to detect aquatic systems pollution. These studies also revealed different levels of tolerance/sensibility facing contamination for the studied species: *Eolimna minima* and *Nitzschia palea* appeared to be more tolerant. The results showed that the development of molecular tools and more precisely the biomarkers is an asset to assess organisms' contamination and water quality.

TU147

Toxic Detection of Mine Water by Recombinant Saccharomyces Cerevisiae with a lysosomal Specific Biomarker

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Lysosome which has various enzymes inside is one of the cell-organelle used as biomonitoring tool in environmental pollution. In this study, the expression of lysosomal proteomic in yeast in response with toxic chemicals, such as sodium meta-arsenite and tetracycline was analyzed for screening specific biomarkers. After that, a recombinant yeast contained this biomarker were constructed for toxic detection in pure toxic chemicals and 7 mine water samples. The results indicated that each chemical had an optimal dose at which the fluorescent protein intensity reached the peak. In the case of water samples, the yeast showed the response with sample 1, 3, 4 and 5; whereas there is no response with sample 2, 6 and 7. In conclude, the recombinant yeast showed a high ability of toxic detection in response with several chemicals such as heavy metals and pharmaceuticals. In the case of mine water samples, the response varied depending on the sample content. **Acknowledgement** This work was carried out with the support of "Cooperative Research Program for agriculture Science & Technology Development (Project title: Development of Target-specific Antimicrobial and Neutralizing Agents for Livestock Biological Hazardous Factors, Project No:PJ01052701)" Rural Development Administration, Republic of Korea. The authors are grateful for their support.

TU148

Proteomic Analysis of Daphnia magna Exposed to Lead (II) Acetate Trihydrate and Atrazine for Screening Potential Biomarker

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Heavy metals and pesticides are main factors causing water pollution via industrial and agricultural waste. However, it still exists a limitation of well understanding about how these toxics effect the aquatic organisms, expecially at molecular levels. In this study, acute toxicity tests were performed according to EPA protocol (2002) to assess the impacts of Lead (II) acetate trihydrate and Atrazine on aquatic species using a typical freshwater flea *Daphnia magna*. Besides, proteomic analysis was performed to identify proteins involved in the stress responses of *D. magna* to these toxic chemicals. The lethal concentrations LC20 of each chemical, which were determined by previous acute test, were used for a 24 h exposure to the 21 days daphnids before isolating the total protein. The proteomic profile was examined with a pH range from 3 to 10 using 2-DE method and then analyzed with Progenesis software to explore the differentially expressed proteins (DEPs) compared with control organisms. The results showed that there were some up- and down- regulated proteins in *D. magna* responding to these toxic chemicals. The DEPs are helpful to understand the molecular responses of *D. magna* to Lead (II) acetate trihydrate and Atrazine and can be used as novel biomarker candidates to detect these heavy metal and pesticide. This work was carried out with the support of "Cooperative Research Program for agriculture Science & Technology Development (Project title: Development of Target-specific Antimicrobial and Neutralizing Agents for Livestock Biological Hazardous Factors, Project No:PJ01052701)' Rural Development Administration, Republic of Korea. The authors are grateful for their support.

TU149

Development of Triclonsan Detecting Biomarker Using Daphnia magna

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Triclosan has been used an antibacterial and antifungal agent found in consumer products including toothpaste, soaps, toys and cosmetics. However, it is toxic to aquatic / land organisms, human and environmental. Therefore, there is a need for the development of novel biomarker to monitor the toxicity of the triclosan. In this study, acute toxicity tests were performed according to EPA protocol to assess the impacts of triclosan on the typical freshwater flea *Daphnia magna*. The lethal concentration for 20%(LC20) of triclosan for *D. magna* was determined to be 300μg/L. Additionally, the proteomic profile of treated *D.magna* LC20 was also analysed using two-dimensional(2D) electrophoresis technique. The comparison in protein expression pattern between control and TCS-treated organisms was then carried out using Progenesis software to explore the differentially expressed proteins(DEPs). After that, MALDI-TOF analysis was also conducted to identify the proteins dots of interest selected from 2D gels. The changed protein spots due to triclosan treatment can be used as novel biomarker candidates to detect triclosan as being mentioned above. From MALDI-TOF results, certain promoters that can express certain DEPs were inserted upstream to the green fluorescent protein of plasmid vector. Then plasmid transfection was carried out on Hela cell to confirm the working ability of these promoters. The changed protein spots due to TCS treatment can be used as novel biomarker candidates to detect triclosan as being mentioned above. This work was carried out with the support of "Cooperative Research Program for Agriculture Science & Technology Development (Project No: PJ01051502)" Rural Development Administration, Republic of Korea. The authors are grateful for their support.

Interactive effects of climate change and contaminants: environmental risks and human health implications (P)

TU150

Methylmercury (MeHg) and climate change: neurophysiologic and behavioral responses of Senegalese sole Solea senegalensis

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Methylmercury (MeHg) is an extremely hazardous pollutant. Consequences of MeHg exposure on biota in a climate change context are still unknown. Acetylcholinesterase (AChE) hydrolyzes acetylcholine, a prominent neurotransmitter in all vertebrates, both in the sympathetic and parasympathetic nervous systems. Lateralization produces faster and more efficient responses to external stimuli. *Solea senegalensis* use the bottom as camouflage, thus disruption in bottom-choosing ability and lateralization may prove ecologically disastrous. The brain is divided in morphologically different neural substructures, each playing different roles in neurotransmission and MeHg accumulates at different rates on each substructure. We assessed the effects of joint exposure to MeHg contamination and climate change variables in *S. senegalensis* behavior processes, as well as acetylcholinesterase activity and different brain regions weight (forebrain, optic tectum, cerebellum and brainstem). During 28 days**,** *S. senegalensis* specimens were maintained under three factor crossed treatments: MeHg contaminated feed, pH (ambient CO_2: 8.0, high CO_2: 7.6), and temperature (ambient: 19ºC, high: 23ºC). Behavior was assessed by calculating absolute and relative lateralization using a detour test, and by habitat preference, measuring time spent between two habitats: simple and complex (all tests, n=10). Neural substructures were separated, weighed individually, and AChE was assessed in each region. Relative weight for each was plotted and a linear regression from the control was calculated. MeHg intake correlated with increased time spent in complex habitat, where fish could not camouflage or hide efficiently. In addition, MeHg reduced lateralization on each fish, leading to complete loss of lateralization at populational level. Acidification also led to increased time spent in complex habitat, only in non-contaminated treatments. All four brain regions registered differences in relative weight under different stressors. Optic tectum relative weight decreased under all stressors, whereas other brain regions were increased. AChE data is being analyzed. Climate change variables were as important as MeHg in altering brain region relative size, but were downplayed at an ecological level. Disruption of important behavior processes along with deregulation of neural substructure functions, may lead to decreases in *S. senegalensis* ecological fitness.

TU151

Can bivalves recover from short-term exposure to environmental changes? The combined effect of salinity shifts and Arsenic contamination

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Arsenic (As) is one of the most toxic elements in the environment, especially when in its inorganic form, being listed as priority hazardous substance in the world. Although many authors have investigated the levels of As in sediments and water, and As bioavailability and bioaccumulation patterns in the marine environment, less have addressed the toxicity of this metalloid namely in marine bivalves. Besides As, salinity is also a stress factor for aquatic environments since it can change across spatial and temporal scales, species richness, abundance and spatial distribution but also individuals health status. It is foreseeable that climate changes, predicted for the next 100 years, will cause salinity shifts in estuarine and coastal areas. Beyond this long-term environment alteration, extreme weather events are becoming more frequent which can also lead to salinity fast changes. Salinity changes are also expected to change the sensitivity of aquatic organisms namely to metals and metalloids, due to alterations on their physiological and biochemical characteristics. Salinity changes may also alter the toxic capacity of contaminants by changing their chemical speciation, solubility and adsorption, with consequences to their bioavailability. Thus, the current study assessed the biochemical alterations induced in the clam species *Ruditapes philippinarum* after exposure to salinity shifts (14, 28 and 42 g L^{-1}) and As contamination (0 and 2 mg L^{-1}). The capacity of this species to recover (96h and 28 days) after exposure to both stressors, acting alone and in combination, was also evaluated. After exposure, regardless the salinity tested, clams contaminated with As showed higher concentrations than non-contaminated specimens. After recovery, As concentration in clams decreased, with contaminated and non-contaminated specimens presenting similar values. The results obtained further demonstrated that exposure to As (2 mg L^{-1}) at different salinities (salinities 14, 28 and 42 g L^{-1}) and salinity 42 g L^{-1} (As 0 mg L^{-1}) lead to an increase of lipid peroxidation and detoxification mechanisms in clams, compared with clams non-contaminated under salinities 14 and 28 g L-1. After recovery, at salinities 14 and 28 g L^{-1}, clams previously exposed to As were capable to decrease their oxidative stress to levels found in non-contaminated clams. Nevertheless, at salinity 42 g L^{-1} both contaminated and non-contaminated clams did not survive.

TU152
Transcriptomic response of the aquatic plant Elodea nuttallii to mercury and ultraviolet radiation
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We aimed to investigate the influence of enhanced UV radiation on the response of the aquatic plant *E. nuttallii* to interacting stresses. In the present work we exposed *E. nuttallii* to UV (0.55 Wm^{-2} UV_{BE}) for 6 h and to Hg for 24 h or to both for combined treatment. We analysed Hg content, oxidative stress response, effect on pigment content, as well as the transcriptome. UV radiation decreased Hg uptake in shoots as compared to plants exposed to Hg alone. Pigments tended to decrease in response to UV and Hg, and a cumulative effect of combined treatment was observed. Looking at oxidative stress enzymes, we observed an opposite effect of combined treatment: peroxidase activity was significantly decreased by UV and Hg treatments alone, whereas a combination of both abolished this effect. Results of RNA-Seq confirmed results obtained from analysis of pigments and stress response. At the transcriptome level, in terms of stress response, combined exposure had more severe effects than Hg or UV single exposures. Briefly, we found genes involved in sugar metabolism and oxidative stress to be up-regulated, and many photosynthesis-related genes to be down-regulated by combined Hg and UV exposure. In conclusion, we were able to show that UV exposure influences accumulation and tolerance to Hg.

TU153
Combined effects of temperature and cadmium on membrane phospholipid composition, aerobic and antioxidant capacities and lipid peroxidation in two freshwater fishes
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In ectotherms, temperature acclimation is always accompanied by membrane fatty acid remodelling and modifications of aerobic and antioxidant capacities. However, metal exposure can alter the capacity of organisms to cope with stressful conditions. In this context, this study investigated the combined effects of temperature and cadmium (Cd) exposure on membrane fatty acid composition, aerobic capacity (determined by the activities of cytochrome c oxidase (CCO) and citrate synthase (CS)), antioxidant enzymes activity (superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx)) and lipid peroxidation (malondialdehyde (MDA) concentration). Two freshwater species, the fathead minnow (*Pimephales promelas*) and the yellow perch (*Perca flavescens*), were acclimatized at two different temperatures, 15?C-30?C and 9?C-28?C respectively, for eight weeks in the presence or absence of Cd (8ug /L). At low temperatures, the membranes of two species were enriched in polyunsaturated fatty acids (PUFA) while the proportion of saturated fatty acids (SFA) decreased, a well-established homeoviscous response aimed at maintaining membrane fluidity. This normal response was altered in the presence of Cd. In yellow perch muscle, the proportion of PUFA decreased at 9?C and increased at 28?C compared to control fish at the same temperatures. In the muscle of Cd-exposed fathead minnows, PUFA increased at 28?C but was not modified at 15?C. In the latter species, CCO activity was increased by two-fold at 9°C compared to 28°C but unaffected by Cd. In contrast, in yellow perch, CCO activity was not affected by temperature but it was inhibited by Cd. Antioxidant enzymes (SOD, CAT and GPx) reacted differently between the two species to the two stressors but MDA concentration was consistently higher under cold conditions. The activity of CCO was strongly correlated with PUFA percentage, especially in fathead minnows. The dominant PUFA were arachidonic, eicosapentaenoic and docosahexaenoic acids. These fatty acids are among the major constituents of cardiolipin. Consequently, we hypothesize that the observed modifications in CCO activity were mostly due temperature or Cd-induced modifications of fatty acids surrounding this enzyme. Our study demonstrates that physiological responses to thermal acclimation can be affected by metal exposure, highlighting the importance of studying the interaction of multiple stressors.

TU154
Levels and trends of contaminants in human populations in the Arctic
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The Arctic Monitoring and Assessment Programme (AMAP) is one of six working groups (WG) established under the Arctic Council. AMAP is tasked with monitoring the levels of contaminants present in the Arctic environment and people as well as assessing their effects on a continuous basis, and reporting these results regularly. This presentation provides an overview of the human biomonitoring data from all eight Arctic countries reported in the 2015 Human Health Assessment Report. Levels of contaminants are declining in the monitored Arctic populations, but not consistently across the Arctic. Certain populations are experiencing more rapid declines than others, and certain populations have concentrations that are remaining stable or are still increasing. Most Arctic populations described in this chapter continue to experience elevated levels of these contaminants compared to other populations monitored worldwide, for example, mercury, where 7 to 85% of Inuit women 18 to 39 years of age in Arctic Canada and Greenland exceed the Canadian provisional blood guidance value of 8 ug/L established for children and women of childbearing age. There are certain contaminants, like perfluorinated compounds (PFCs) and polybrominated diphenyl ethers (PBDEs) which are still increasing in Arctic populations, and require more investigation to find the predominant and important sources of exposure. Most of these data have been collected over the last twenty years and are from all 8 circumpolar countries. Coordinated, international biomonitoring must continue in the future to determine if levels of these contaminants, and others, are changing in Arctic populations. This work continues to support international risk management efforts under the Stockholm Convention. This work also supports the objectives of Canada's Chemicals Management Plan.

TU155
Ecophysiological responses of Gammarus pulex to the combined effects of temperature and ammonia
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In freshwater ecosystems, accumulation of pollutants mostly results from anthropic activities. Today, it is a worrying issue due to potential drastic consequences on aquatic biodiversity . Ammonia appears among compounds that may act as a threatening contaminants for many species. In water, ammonia concentration heavily depends on both temperature and pH. Therefore, we investigated the effects of temperature (10, 15, 20 & 25°C), ammonia concentration (0, 0.5, 1, 2, 3 & 4 mg/L), and their combination, on the survival ability and the molecular responses of *Gammarus pulex*, a common freshwater crustacean. Survival experiments revealed an unexpected trend toward an antagonistic interaction between the two parameters. While a week of exposure to 25°C induced a 43% reduction of survival in the absence of ammonia, the addition of a high ammonia concentration (4mg/L) blurred the stressful effect of the temperature. Indeed, in the 4 mg/L condition, the survival was 10% higher at 25°C than in the 10°C condition. The analysis of survival data through Bayesian toxico-kinetics/toxico-dynamics (TK-TD) models confirmed this antagonistic interaction. Between the 10°C and the 25°C exposure treatments, ammonia Lethal Concentration for 50% of individuals (LC_{50}) estimates increased from 2.56 to 5.4 mg/L and ammonia No Effect Concentration (NEC) estimates increased from 0.90 to 1.25 mg/L respectively. Using qPCR techniques, we also tried to associate these survival patterns to variations in the expression of the *hsp70* gene, a generic biomarker of stress. Gammarids exposed to 25°C showed a 14-fold change overexpression of *hsp70* mRNA compared to controls, suggesting that this temperature was stressful. *Hsp70* was not induced at the other temperatures. The *hsp70* overexpression at 25°C was unrelated to ammonia concentration so that ammonia-temperature interaction was not found. Our results highlight the influence of temperature and ammonia concentration as crucial environmental parameters for the physiology of *G. pulex*. Developing new and alternative molecular biomarkers will help to monitor and decipher the biological response of natural populations potentially exposed to both stress. Keywords : ammonia, heat shock proteins, freshwater crustacean, TK-TD models

TU156
OA-RESPONSE: Ecosystems in a changing ocean - New CO2 and temperature manipulation system for use in ecological assessment of food web functioning under multiple stressor scenarios
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The decline in pH and rise in CO_2 manifested through ocean acidification (OA) could potentially result in significant physiological effects on microbes, plankton, and larger organisms, and lead to alterations in biogeochemical cycles and ecosystem function. Concurrent with OA, a variety of other physical and chemical changes are projected for coastal regions that could affect organisms, including increases in the quantity of UV light, increases in sea surface temperature, reduction in salinity, and increases in emerging contaminants e.g. human and veterinary medicines. Phytoplankton and zooplankton comprise the basis of productive coastal and open ocean food webs and provide valuable ecosystem services that humans are socioeconomically dependent on (e.g. animals from fish larvae to baleen whales are dependent on plankton for nutritional needs). The phytoplankton and copepod species (*S. costatum*, *R. baltica*, and *T. battagliai*) that were investigated in terms of response to OA/multiple global change variables are considered vital/keystone to coastal Arctic and Norwegian ecosystems and are the focus of this paper. Two distinct but complimentary systems for the manipulation of seawater CO_2 for use in OA experiments at the "micro-scale" and a more traditional "macro", litre-scale system were developed. For the micro-scale system *T. battagliai* was used as a model organism. There has been documented resilience of copepods to acidification (due to proteins and chitin) and they have a greater capacity to adapt to climate change. However, other factors such as changes in temperature have proven to affect various life stages. In addition exposure to high CO_2 levels can enhance the sensitivity of organisms to thermal extremes. Furthermore, changes in salinity may affect organisms as an independent stressor as well as by altering the bioavailability and in some instances increasing the toxicity of chemicals. The two systems were successfully developed for use in OA experiments, the micro-scale system showed great potential to investigate multiple stressors. In general there is a misconception that zooplankton are insensitive to OA, however, employing more sensitive endpoints like naupliar development and reproduction showed responses vary between life stages. Reproduction and development were greatly influenced by the modification of environmental parameters such as pH, salinity and temperature, which also influenced the toxicity of known toxicants to *T. battagliai*.

TU157

Combined effect of metallic pollutants (Cu and Ag) and temperature on the early life stages of the Mediterranean mussel Mytilus galloprovincialis

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The present work aimed to assess the effect of metallic pollutants (Cu and Ag) exposure along with temperature rise, on early life stages of the Mediterranean mussel Mytilus galloprovincialis. For this, embryo-toxicity assay and RT-qPCR were used to evaluate developmental effectsand gene expression modulation in D-larvae exposed to both thermal and chemical stress. At first, mussel embryos just fertilized were exposed to rising concentrationsof copper (Cu) (0.5 – 500 μg/l) and silver (Ag) (0.1 – 100 μg/l) along with temperature gradient (18, 20, 22 or 24°C) in order to characterize their toxicity and determine the EC50 of each toxicant for the different tested temperatures. Then, increasing concentrations of a Cu-Ag mixture were applied in order to understand and assess the mixture effect at different temperatures. Embryotoxicity was measured after 48h (stage D-larvae) by considering both the percentage of abnormalities and development arrest in D-larvae. Eighteen target genes involved in antioxidant defense, DNA repair, apoptosis, proteolyse, transcription, thermal stress and metalic detoxification were investigated in 48h-developpement larvae. Those were exposed to Cu EC20 (9.6 μg/l), Ag EC20 (2.3 μg/l) and the mixture of (Cu EC10 (6.7 μg/l) +Ag EC10 (1.3 μg/l)) at 3 temperatures (18, 20 or 22°C). The results suggest that the best temperature for mussel larvae development is 18°C (≈ 10% malformations). The rate of malformations increases with increasing temperature to reach 100% at 24°C. Silver has been proved to be more toxic than copper with a half maximal effective concentration (EC50) at 18°C of 6.20 μg/l (5.09 – 6.72) and 18.14 μg/l (13.23 – 18.26) respectively. Temperature seems to increasethe toxicity of these metals as proved with the EC50 obtained at 20°C \nrespectively for Cu and Ag 16.3 μg/l (12.01-16.42) and 3.7 μg/l (2,81-4,07). The higher toxicity of AgCu mixture suggests slightly antagonistic effect ofthe two metallic ions with a synergy factor of 0.68 at 18 ° C. Significant gene transcription modulation was observed for several genes involved in thermal stress (hsp 70), proteolyse (catepsin), DNA repair (p53) and anti-oxidant defense (cat). These changes in gene expression provide new insights to better understand sensitivity of mussels early life stages to combined effects of metallic pollution and temperature rise.

TU158

Did intertidal biota decline after the 2011 Great East Japan Earthquake and Tsunami and the Fukushima nuclear disaster?

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In 2011, 2012, and 2013, in the intertidal zones of eastern Japan, we investigated the ecological effects of the severe accident at the Fukushima Daiichi Nuclear Power Plant that accompanied the 2011 Great East Japan Earthquake and Tsunami. The number of intertidal species decreased significantly with decreasing distance from the power plant, and no rock shell (*Thais clavigera*) specimens were collected near the plant, from Hirono to Futaba Beach (a distance of approximately 30 km) in 2012. The collection of rock shell specimens at many other sites hit by the tsunami suggests that the absence of rock shells around the plant in 2012 might have been caused by the nuclear accident in 2011. Quantitative surveys in 2013 showed that the number of species and population densities in the intertidal zones were much lower at sites near, or within several kilometers south of, the plant than at other sites and lower than in 1995, especially in the case of Arthropoda. There is no clear explanation for these findings, but it is evident that the intertidal biota around the power plant has been affected since the nuclear accident.

Combining exposure and effects models and data for landscape based risk assessment in a regulatory context (P)

TU159

Effect modelling as a bridge from the first tier to the landscape level: An example for mammals

M. Wang, WSC Scientific GmbH / Dept Efate Modelling; M. Foudoulakis, Dow Agrosciences / RSRA ERS; S. Norman, RidgewayEco; N.N. Poletika, Dow Agro Sciences LLC / Field Exposure and Effects Department

In the present study body burden modelling is used to predict potential effects of a pesticide application in mammals. The study is based on a substance with a rather worst-case first tier profile. For parameterisation of the body burden model purpose-designed studies were conducted and the model was validated using data from independent studies, covering different species. The validation demonstrated that the model provided robust but slightly conservative results. Body burden modelling was then conducted considering realistic feeding behaviour obtained from the literature and measured residues in food items. Results indicated a low risk in all focal species. These results were in line with data from large-scale field studies from different crops, which indicated no effects on population abundance or reproduction. This risk assessment exemplarily demonstrates how effect modelling can help to extrapolate results from the lower tier, which only facilitates a limited view of the potential risk since not all factors determining the risk are considered, to a real field situation. The gained knowledge of all factors, including mode of action, individual behaviour or ecology allow a much better understanding of risk and to draw conclusions about the acceptability of a pesticide use with much more certainty.

TU160

Pesticide treatment regimes in landscape based risk assessment

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In order to develop landscape based risk assessments it is essential to know all the different stressors, including pesticides, which can potentially impact populations of non-target organisms in the different agricultural landscapes of Europe. The environmental risk assessment evaluates the risk for one single substance at a time. This makes it difficult to apply the recovery principle in the risk assessment. In order to have a correct estimate of the risk to non-target organisms it is essential to link effects to real exposure to pesticides in agricultural landscapes. The European Food Safety Authority (EFSA) recently published a technical report on pesticide application data representative of different crops and different regulatory zones in Europe which can be used as a basis for a future landscape based risk assessment. A total of 394 farms and 2814 fields were surveyed covering the following Northern (Lithuania), Central (Belgium, Netherlands, Poland and United Kingdom) and Southern (Greece, Italy and Spain) zones. Data were collected for arable crops, orchards and grape vines. For 580 fields detailed information on the field margins and surrounding structures was collected and for these fields in some countries detailed historical pesticide usage data, for up to five years, was also collected. The number of products and substance classes applied and the number of spray applications within one growing season varied enormously among the different crops (e.g. 25 spray applications and 39 different formulations in apple orchards versus 5 spray applications and 14 formulations in sugar beet). This suggests that the exposure and hence risk for non-target organisms may vary to a large extent depending on the crops which are grown in different agricultural landscapes. Therefore the development of environmental scenarios including crop relevant treatment regimes are essential for a realistic landscape level risk assessment approach.

TU161

From the first tier to the landscape: How modelling helps to understand the risk

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In pesticide risk assessment first tier calculations are thought to provide an efficient screening method to detect substances that may need a more detailed evaluation. The first tier risk assessment is usually viewed as a method indicating the level of risk of a given toxic substance. Being based on toxicity and exposure only, other factors that may potentially play an important role in determining the risk to individual animals, are not included. On the other end of the scale, data from large scale field studies may reveal information of the real risk on the landscape level and under real field conditions. Quite often, it is important to understand the results in the field from actual use to reach evidence-based decisions while in some cases it may be difficult for risk assessors to interpret contradicting results. In such cases, effect modelling helps to understand results in the field or why results of the first tier and the landscape scale are contradictory, by making it possible to understand the mechanisms that determine the real risk in a real agricultural setting. In the present study, an example of a substance with a rather worst-case first tier profile is presented, for which information from the field scale indicates a low risk in birds. Body-burden modelling was conducted with fit-for-purpose laboratory studies to understand why no effects were detected on the landscape scale, while the first tier indicated a risk. This evaluation helped to determine the factors that mostly affected the risk and that were not covered by the first tier risk assessment. This example shows how effect modelling can provide a bridge between the first tier and the field scale, and how both regulators and industry can find the evidence and benefit from a better understanding of the mechanisms determining or preventing a risk.

TU162
Models' output on how to combine spatiotemporal scales, biological organizational levels and interactions in environmental risk assessment: A short review.
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This literature review chronicles the modelling approaches that have been developed for assessing potential risk in the context of environmental risk assessment for pesticides with the aim to reflect the up-to-date outcomes on how to combine spatiotemporal scales, biological organizational levels and interactions in a regulatory context. For this purpose the literature review was carried out by using keywords in all fields of the “Expert search” option “Journals” of ScienceDirect with the formula “environmental risk assessment” and “exposure” and “effect” and “model” and “pesticide”. From 83 publications a thorough full text examination was conducted to include only the publications with consistent information. Therefore only publications that studied and proposed the development, combination and/ or improvement of models to be used in environmental risk assessment were incorporated in this review’s database. Thus data from a corpus of 22 publications from 1997 to 2015 were considered in the results of the current review. Different biological organizational levels were included from individual to populations to community up to landscape. Most models have been initially developed for aquatic organisms and environments though a small minority for soil organisms. Although toxicokinetic parameters have already been mostly developed for pesticides, they were not included in the majority of the proposed models and approaches in the reviewed publications to link exposure with effect. Therefore a clear picture on their contribution to predict dynamic responses over space and time under different exposure regimes cannot be regarded. This applies even for the studies proposing a combination of individual- and population-based models. Population dynamics are considered in either effect or exposure models as well as their combinations. Biological interactions such as predation, competition and density dependence, interactions between different trophic levels, synergism, and also interactions between individuals and their environments from which population dynamics emerge were studied in approximately half of the reviewed database.

TU163
Investigating an ecosystem services framework to advance chemical risk assessment and risk management
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Our recent review of current EU legislation identified that environmental protection goals tend to be poorly defined, leading to uncertainty in how to achieve such protection. As there is growing interest towards integrating the assessment of ecosystem services (human well-being benefits that flow from natural capital) into decision-making processes well defined protection goals are required to enable risk assessments to be extended to encompass ecosystem services. Recently, the European Food Safety Authority (EFSA) developed a framework to identify ecosystem services potentially affected by (agro)chemicals, set specific protection goals (SPGs) and assess relevant risk assessment schemes. We have investigated the applicability of the EFSA framework for developing SPGs for a wide range of other chemicals using four case studies spanning a range of different emission scenarios and receptor habitats. Based on these case studies, we have discussed and presented approaches by which the EFSA framework’s applicability could be extended to the wider chemical industry. The selected case studies chosen included: oil refinery waste water exposure in estuarine environments; (ii) oil dispersant exposure in aquatic environments; (iii) down the drain chemicals exposure in a wide range of ecosystems (terrestrial and aquatic); (iv) persistent organic pollutant exposure in remote (pristine) environments. A five-step process was followed to identify habitats and ecosystem services potentially impacted by the chemical emissions described and subsequently define possible SPGs in each case study. Case studies demonstrated that it is possible to apply the ecosystem services concept to derive SPGs for a broad range of chemical exposure scenarios. By identifying key habitats and ecosystem services of concern, the approach offers the potential for greater spatial and temporal resolution, together with increased environmental relevance, of risk chemical risk assessments. With modifications including improved clarity on terminology/definitions and further development/refinement of the key concepts, we believe the principles of the EFSA framework could provide a methodical approach to the identification and prioritization of ecosystems and services which are most at risk from chemical exposure.

Recent Developments and Current Issues in Bioaccumulation Assessment (P)

TU164
Using in vitro assay based critical input parameters to evaluating a fish bioaccumulation model for ionogenic organic substances (BIONIC)
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Regulatory and scientific interest in the bioaccumulation and potential ecological risk of ionizable organic chemicals (IOCs) is increasing. Most modeling tools were developed for neutral organic chemicals rather than IOCs. The main objectives of the LRI ECO21 project were to improve methods and data (i.e., membrane-water partition coefficients and biotransformation rates) required to parameterize a mechanistic fish bioaccumulation model for IOCs and to evaluate the performance of key aspects of the model (e.g., gill uptake & biotransformation). *In vitro* biotransformation assays derived from trout liver S9 homogenates were conducted for 50 IOC structures, covering 8 different types of acids and bases. The *in vitro* rates are being compared with *in vivo* and QSAR estimates. New QSARs have been developed for membrane-water partition coefficients for ionized compounds.

TU165
QSAR prediction of human Biotransformation Half-Lives: models development and application
E. Papa, Department of Theoretical and Applied Sciences; A. Sangion, P. Gramatica, University of Insubria / DiSTA
The evaluation of bioaccumulation potential is a key point in the risk assessment of chemicals. However, the quantification of the various processes that characterise bioaccumulation, such as uptake, metabolism and excretion, is challenging due to the extensive costs and the time required to perform bioaccumulation testing. In the last years efforts have been made to generate models based on Quantitative Structure-Activity Relationships (QSARs) in order to predict bioaccumulation-related parameters (i.e. bioconcentration factors, primary biotransformation rate constants and corresponding half-lives) from the molecular structure of compounds. These tools are useful to reduce the experimental impact, and to predict the potential behaviour of existing and not yet synthesised chemicals at a preliminary screening phase. In this poster, we present different QSAR models, which were generated for the prediction of biotransformation half-lives (HL; days) measured in humans, starting from 5 literature data sets and over 1000 organic compounds. The models were developed by paying particular attention to statistical robustness and external predictivity, applicability domain and interpretability. Multiple Linear Regression (MLR-Ordinary Least Squares(OLS) method) and the Genetic Algorithm Variable Subset Selection procedure were performed by using the software QSARINS, and including as independent variables the theoretical molecular descriptors calculated from molecular structures by the software PaDEL Descriptors. These models were applied to predict the potential for human biotransformation of over 1100 Pharamceuticals and Personal Care Products (PPCPs). Additionally, previously published models were applied to predict the biotransformation potential in fish, and the potential behaviuor of the 1100 PPCPs as Persistent, Bioaccumulative, and Toxic compounds (i.e. PBTs). All the data predicted by the aforementioned models were combined by multivariate analysis (Principal Components Analysis, PCA). The analysis allowed for the distinction of the PPCPs slowly biotransformed among those with potential PBT behavior. These PPCPs may be of higher human and environmental concern than PPCPs that are more easily biotransformed. Results from this study show that QSAR models are useful tools not only to fill data gaps but also to refine previous assessments, such as the potential PBT behaviour.

TU166
Determination of thresholds in marine mussels as alternative to

Environmental Quality Standards in marine water: study of bioaccumulation (BAF) and bioconcentration (BCF) factors

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The Water Framework Directive (WFD) establishes Environmental Quality Standards (EQS) in marine water for 34 priority substances. Among these substances, 25 are hydrophobic and bioaccumulable (2 metals and 23 organic compounds). For these 25 substances, monitoring in water matrix is not appropriate and an alternative matrix should be developed. Bivalve mollusks, particularly mussels (*Mytilus edulis, Mytilus galloprovincialis*), are used by Ifremer as a quantitative biological indicator since 1979 in France, to assess the marine water quality. This study has been carried out in order to determine thresholds in mussels at least as protective as EQS in marine water laid down by the WFD. Three steps are defined: - Provide an overview of knowledges about the relations between the concentrations of contaminants in the marine water and mussels through bioaccumulation factor (BAF) and bioconcentration factor (BCF). This allows to examine how a BCF or a BAF can be determined: BCF can be determined experimentally (according to US EPA or ASTM standards), or by Quantitative Activity-Structure Relationship models (QSAR): four equations can be used for mussels. BAF can be determined by field experiment; but none standards exists. It could be determined by using QSAR but this method is considered as invalid for mussels, or by using existing model: Dynamic Budget Model, but this is complex to use. - Collect concentrations data in marine water (C_{water}) in bibliography for those 25 substances ; and compare them with concentration in mussels ($C_{mussels}$) obtained through French monitoring network of chemicals contaminants (ROCCH) and biological integrator network RINBIO. According to available data, this leads to determine the BAF or the BCF ($C_{mussels}$ /C_{water}) with field data. - Compare BAF and BCF values (when available) obtained with various methods for these substances: BCF (stemming from the bibliography, using experimental process), BCF calculated by QSAR and BAF based on field data. This study points out that experimental BCF data are available for 3 substances (Chlorpyrifos, HCH, Pentachlorobenzene). BCF by QSAR can be calculated for 20 substances. The use of field data allows to evaluate 6 BAF: 4 for organic compounds and 2 for metals. Using these BCF or BAF value, thresholds in shellfish can be determined as an alternative to EQS in marine water.

TU167

A reference database for bioaccumulation of organic chemicals in aquatic and terrestrial invertebrates

C.C. Chen, City University of Hong Kong / Department of Architecture and Civil Engineering; D. Kuo, City University of Hong Kong / Architecture and Civil Engineering

Recent advances on the bioaccumulation of organic compounds in fish have been made possible by the availability of high quality bioaccumulation and biotransformation databases. Despite the many bioaccumulation studies made for aquatic and terrestrial invertebrates over the last few decades, and the incorporation of invertebrates into standard bioaccumulation testing protocols, a comprehensive and quality bioaccumulation database of organic compounds in invertebrates has not been available. In order to meet this research need, we have commenced in reviewing and compiling bioaccumulation metrics reported for both soil and benthic invertebrates. Standard bioaccumulation metrics (i.e., BCF, BAF, BSAF, etc.), kinetic rate constants for specific uptake and dissipation processes, details on exposure conditions and experimental setup have been gathered, classified, and archived from primary experimental literature. Target chemicals included both non-polar organic and ionogenic/ionizable organic compounds. Target invertebrate organisms included worms, bivalves, insects, amphipods, isopods, gastropods, decapods, and asteroids, etc. Secondary sources of information – existing bioaccumulation databases or technical summaries – that do not have adequate details and/or show of data points were used only as further sources of reference. To date, a total of over 4000 data entries have been gathered for worms, and approximately 2000 data entries have been collected for other non-worm invertebrates. Individual data entries were reviewed for their quality according to the experimental setup and protocol adopted as well as the data reduction method used, following the approach/scheme outlined by Klimisch et al. (1997) and Arnot & Gobas (2006). Preliminary analysis of the existing data suggested a few interesting trends in the bioaccumulation data. Further scrutiny and analysis of the collected data may reveal meta-level knowledge with respect to bioaccumulation of organic pollutants. The completed version of this database will provide a solid starting point for understanding and modeling the bioaccumulation of organic contaminants in soil and sediment.

TU168

A field monitoring programme to assess the potential for bioaccumulation and biomagnification of a plant protection product in the aquatic and terrestrial environment

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As part of the regulatory approval under Regulation (EC) No 1107/2009, a field monitoring programme was conducted following real-world applications to assess the potential for bioaccumulation and biomagnification of a plant protection product in the aquatic and terrestrial environment. The main objective of this presentation is to review what we have learned through direct experience with regards to the sample number requirements, the sampling techniques and the field study design during the conduct of the monitoring programme. The study design was set up to investigate the spatial and temporal fate of the test item throughout terrestrial food chains for soil and crop and aquatic food chains within water and sediment. Large scale field sites (up to 24 ha) were selected according to worst-case conditions, especially in terms of aquatic exposure – e.g. adjacent water bodies. A combined sampling of terrestrial specimens (field soil, crop vegetation, earthworms, small mammals (e.g., common voles and common shrews)) was conducted over periods of up to six months to characterise the magnitude and duration of residues in the relevant terrestrial compartments. To achieve comparable data throughout different matrices and to control spatial variations, replicates with randomized subplots within the treated plot were set up for collecting soil characterisation, soil residue, earthworm residue and crop residue samples from co-located sampling areas. To assess the exposure of the aquatic system in the relevant aquatic compartments, pond water, run-off water, aquatic sediments, aquatic vegetation, macroinvertebrates and fish were sampled from well distributed sampling areas. Sampling was performed from up to 30 replicates and with an abundant amount of samples per event to obtain statistically representative data. Futher in assessing the potential for bioaccumulation and biomagnification, the following matrices were analyzed separately for residues: soil from soil cores, surface and deep burrowing earthworms, oilseed rape plants, oilseed rape leaves, oilseed rape litter, small mammals without gut and gut content, small mammal guts and small mammal gut contents, water, sediment, aquatic macrophytes, macroinvertebrates and fish without gut and/or mucus, fish gut and fish mucus. To be able to compare the residues of different matrices, soil and plant residues were normalized for organic carbon; residues of earthworms and small mammals were normalized to lipid content.

TU169

Results and interpretation of a field-scale bioaccumulation and biomagnification monitoring programme

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Bifenthrin was recently approved as an active substance, in accordance with Regulation (EC) No 1107/2009, with a confirmatory data requirement to submit: "*a monitoring programme to assess the potential for bioaccumulation and biomagnification in the aquatic and terrestrial environment*". No guidelines are available for conducting monitoring programmes to assess the potential for bioaccumulation and biomagnification in the environment. Direct spray studies were conducted in North Germany to determine whether bioaccumulation or biomagnification occurred following realistic exposure of aquatic and terrestrial systems to bifenthrin. The investigations were designed to represent the full proposed GAP for bifenthrin in the EU, which includes application to early and mature crops, and to address worst-case scenarios in terms of exposure routes in the environment. In the terrestrial environment, appropriate food chains for soil (soil, earthworm, small omnivorous and insectivorous mammal) and crop (crop, small herbivorous mammal) were sampled. For the aquatic environment, food chains based on water and sediment with aquatic macroinvertebrates and fish were sampled. The mitigation measures employed to ensure acute and chronic safety to aquatic organisms (20 m no-spray buffer and 90% drift reducing nozzles) ensured that bifenthrin residues in the aquatic systems were low, even following purposefully exaggerated irrigation. Detectable residues in fish surface mucus samples demonstrated the presence of bifenthrin in the aquatic system. Despite exposure, however, there was no evidence for bioaccumulation or biomagnification of bifenthrin in aquatic systems. Residues in small mammals were low. Shrews had lower residue levels than earthworms. Other small mammals likely to consume earthworms also had residues below the levels in earthworms and crop, and significantly decreased over time. Residues in voles, which feed on vegetation, were also low and dissipated rapidly. These investigations, involving real-world applications of bifenthrin according to proposed GAP, demonstrated that bifenthrin does not bioaccumulate or biomagnify in aquatic food webs, nor does it biomagnify at the small mammal trophic level in terrestrial systems.

TU170

Application of equilibrium and mechanistic models to facilitate the interpretation of a terrestrial field bioaccumulation study

J.M. Armitage, University of Toronto - Scarborough / Physical and Environmental Sciences; J.A. Arnot, ARC Arnot Research & Consulting / Department of Physical Environmental Science; F. Kröger, Eurofins Agroscience Services GmbH; A. Lau, S. Lakaschus, Eurofins Agroscience Services Chem GmbH

As part of the regulatory approval of bifenthrin as an active substance under

Regulation (EC) No 1107/2009, a monitoring programme to assess the potential for bioaccumulation and biomagnification in the aquatic and terrestrial environment following field application was required. The main objective of this presentation is to demonstrate how equilibrium-based and mechanistic bioaccumulation models can facilitate the interpretation of monitoring data collected during such studies. The focus of the modelling is on the bioaccumulation of bifenthrin by earthworms (e.g., *Lumbricus terrestris*, *Aporrectodea caliginosa*). The primary motivation for application of bioaccumulation models was the observed earthworm-soil concentration ratios, which were much larger than expectations based on the properties of bifenthin (hydrophobicity, potential for biotransformation) and previous laboratory-based studies. A key confounding issue with the field data was that the soil concentrations were based on 10 cm deep (homogenized) cores, whereas the chemical was likely distributed primarily in the upper soil layers. For *L. terrestris*, equilibrium-based and mass balance bioaccumulation modelling supports the hypothesis that this species is exposed primarily via ingestion of crop litter, which exhibited larger concentrations of bifenthrin than the bulk soil at the surface and the concentrations reported for the homogenized 10 cm profile. For this reason, it is important to collect data characterizing the properties of the leaf litter over time (e.g., seasonal differences) as well as measuring chemical concentrations. For *A. calignosa*, the modelling supports the hypothesis that concentrations of bifenthrin in material ingested by these earthworms are substantially elevated compared to the measured concentrations for the homogenized 10 cm soil profile. Collectively, the calculations suggest that the measured concentrations of bifenthrin for the homogenized 10 cm soil profile are not representative for calculating biota-soil accumulation factors (BSAFs) and characterizing the bioaccumulation potential of bifenthrin in the field. In addition to facilitating the interpretation of the data collected during the field campaign, the bioaccumulation modelling conducted here provides a clear direction for additional empirical studies (i.e., hypothesis testing) aimed at characterizing the bioaccumulation potential of organic chemicals in agricultural settings.

TU171

Development of Multibox-AQUAWEB Model for Prediction of Trophic Magnification Factors Influenced by Spatial Concentration Gradients, Species Migration, and Field Sampling Design

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Trophic magnification factors (TMFs) integrate all routes of chemical exposure and ecosystem processes to evaluate the bioaccumulation behavior of chemicals in food-webs. However, current food web bioaccumulation models do not consider spatial heterogeneity, species migration, and field sampling design. Thus, a Multibox-AQUAWEB (MBAW) model was developed to calculate chemical concentrations and TMFs by including the aforementioned processes to the existing AQUAWEB model. The MBAW model was coded as a Microsoft Excel 2013 workbook containing three worksheets for input parameters, four worksheets for calculations based on the mass balance equations, and one worksheet for output. The MBAW model allows users to define numerous compartments to specify vertical and/or horizontal concentration gradients in an aquatic system. The model also requires the users to define species composition, structure, and trophic dynamics of the aquatic food web. For species migration, the users can define the fraction of time that each species occupies a particular compartment. This input specifies the distribution of a species to a certain area and the degree to which a species may be present in multi-dimensional space. The model also provides the users with the option to specify the "sampling" location of each species by identifying the compartment(s) from which the species will be collected. This feature provides a method for investigating the effect of sample collection location on the TMF in situations where spatial differences in concentrations exist. Using the inputs and mechanisms in the model, the MBAW model calculates the steady-state (whole body wet weight and lipid-equivalent) chemical concentrations in species in each compartment. Based on the predicted concentrations, TMFs are calculated as the antilog of the linear regression slope of log-transformed lipid equivalent concentrations regressed on trophic position. The TMF can be calculated for various sampling scenarios to investigate the effect of sampling design on the determination of the TMF in areas with significant spatial concentration gradients. The MBAW model provides guidance on both the conduct and interpretation of field bioaccumulation studies and highlights the need for development of detailed protocols for field bioaccumulation studies in aquatic food-webs.

TU172

Development and testing of a physiologically based toxicokinetic model of the rabbit (Oryctolagus cuniculus) using the PK-Sim® software suite

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Measuring the concentration and effect of pesticides on all affected species under all conceivable scenarios is not feasible. Therefore standard laboratory species are tested and the endpoints are translated to focal species for environmental risk assessment. Modeling is becoming increasingly important for evaluating the consequences of pesticides in untested scenarios. Acute toxic effect predictions require knowledge of the relevant compound concentrations in target organs. Physiologically based pharmacokinetic (PBPK) modeling is a well-established methodology for predicting target tissue concentrations based on the absorption, distribution, metabolism and excretion properties of drugs. The approach facilitates the extrapolation of drug PK knowledge gained in pre-clinical species such as rats and dogs to predict the drug PK in humans. For toxic substances, PBPK modeling is renamed physiologically based toxicokinetic (PBTK) modeling. In the widely used PK-Sim® software suite, physiologically based models which enable PBTK modeling are available for typical clinical species but not for the species such as small mammals which are typically considered for risk assessments. Rabbits are the herbivorous field species for which sufficient data is available to develop and validate a physiologically-based model. In this poster we give details of the rabbit model we have developed and implemented in the PK-Sim® software suite. We show that the model can describe the concentration of drugs in the plasma and different organs of the rabbit. In addition, we illustrate how the physiologically based modeling approach can be used to translate toxicokinetic knowledge from one species to another, for example, from a laboratory rat to a rabbit.

TU173

Transfer kinetics of dissolved perfluorooctanesulfonate (PFOS) to a marine sandworm species

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We determined the respiratory uptake and depuration kinetics of perfluorooctanesulfonate (PFOS) in a polychaete sandworm species, *Perinereis wilsoni*, and compared them with those reported for other aquatic animals. The breadth of taxonomic groups covered by bioaccumulation kinetic studies is still limited. Thus the basis for species-extrapolation of bioaccumulation kinetics is yet to be established. Bioaccumulation of perfluoroalkyl acids (PFAAs) including PFOS in aquatic organisms is of interest because consumption of seafood was suggested as a major source of human exposure to PFAAs. Polychaete species in general represent an important path of chemicals present in the marine coastal environment to higher organisms. A 7-day exposure period was followed by a 9-day depuration period. Sandworms were held in cylindrical containers packed with gravels. The water level was controlled daily to mimic the tide. During the depuration period, the sandworms were fed commercial fish food. We used filtered (0.1 μm) natural seawater kept at about 17.1 °C. Sandworms and seawater were regularly sampled and analyzed for PFOS. Wet mass of the sandworms were monitored by weighing randomly selected individuals. The oxygen consumption rate of the sandworm was also measured. The PFOS depuration rate constant, respiratory uptake rate constant, and respiratory absorption efficiency for the sandworm were estimated by fitting the measured concentrations to a first-order kinetic model. The PFOS concentrations in seawater averaged 30 ng/L during the exposure period of the exposure treatment and were negligibly low in the control treatment and the depuration period of the exposure treatment. The PFOS concentrations in the sandworm samples from the exposure treatment increased during the exposure period and decreased afterwards. The estimated value of the PFOS depuration rate constant (0.050 d^{-1}) was similar to those reported for a freshwater oligochaete and for the whole body of several fish species, but one order of magnitude lower than that for an oyster species. Although the value of the PFOS respiratory uptake rate constant (32 L kg-wet^{-1} d^{-1}) was in the same range as for the whole body of several fish species and the oyster, the value of the respiratory absorption efficiency (0.13) was generally higher than those estimated for several fish species. These results suggested a more efficient respiratory uptake of PFOS by the sandworm than by fish.

TU174

Bioaccumulation of Selenium through the food chain: water - Lemna minor - Pomacea paludosa

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Selenium (Se) has been used as a fungicide in sugar cane growth in South Florida for many decades. The long-term use of Se in these environments has led to an increase in Se concentrations in the soil. Under the Comprehensive Everglades Restoration Plan, thousands of acres of agricultural lands will be flooded to create wetland habitats and water reservoirs. Flooding lands will result in Se desorption from soils to water and would affect aquatic organisms. Aquatic plants such as *Lemna minor* (duckweed), inhabitants of these systems, can accumulate Se which then can be transferred via predation to higher trophic levels. The objective of this study is to study how Se transfers through different levels of the food chain (water-duckweed –*Pomacea paludosa* (Florida apple snail). Under standard laboratory conditions, duckweed was exposed to three different concentrations of Se (100 μg/L, 500 ug/L, 1000 ug/L) for 2 weeks. Apple snails were fed with Se contaminated duckweed for 28 days. During the course of the study, apple snails and duckweed were collected at different time intervals for Se analysis. This study found that Se was bioaccumulated from water to duckweed and apple snails. Se concentrations in duckweed increased with Se treatment concentration and ranged from 1 mg/kg dw (control) to 431 mg/kg dw (T3). Concentration of Se in the snail soft tissue (food + viscera) increased with increasing Se duckweed concentration and ranged from 0.77 mg/kg dw (control, day 0) to 142 mg/kg dw (T3, day 21). In general, Se concentration in apple snails increased in the order of shell, food, and viscera. Se concentration in the snail soft tissue also increased with exposure time. Results of this study are in agreement with the results of other studies with copper and zinc and apple snails.

Challenges in environmental read-across and grouping of substances - when fate, bioaccumulation and ecotoxicological properties are similar enough? (P)

TU175

A comparison of the toxicity of hindered phenols and substituted phenyl amines to terrestrial plants and invertebrates in a sandy soil

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A research project was initiated by Environment Canada's Biological Assessment and Standardization SEction (BASS) as part of the Canadian Federal Government's Chemicals Management Plan (CMP), to assess the toxicity and bioaccumulation potential of two groups of organic compounds to terrestrial organisms. The groups of organic compounds (hindered phenols and substituted phenyl amines) were selected based on the expectation of partitioning primarily to soil, varing availability in soil, and based on limited effect data in general. Two representative chemicals from each group were selected for toxicity testing with the intention of using the empirical data for read-across and to fill data gaps within the groups for subsequent risk assessment purposes. The hindered phenol group was represented by 3,6-di-tert-butylphenol (CAS 128-39-2) and 2,2'-thiodiethylne bis[3-(3,5-di-tert-butyl-4-hydroxyphenyl)propionate] (CAS 41484-35-9), while N-phenyl-o-phenylene diamine (CAS 534-85-0) and N,N'-di-2-naphthyl-1,4-phnylenediamine (CAS 93-46-9) were selcted to represent the substituted phenyl amines group. The inherent toxicity of the test substances were assessed through invertebrate (*Folsomia candida* and *Eisenia andrei*) reproduction and plant (*Trifolium pratense* and *Elymus lanceolatus*) definitive tests, using Environment Canada's standardized soil test methods. Effects on *E. andrei* reproduction were then evaluated in order to select a single chemical from each group for bioaccumulation tests. The generated data, with accompanying exposure characterization will be presented, with an evaluation of the read-across potential of these substances to environmental risk assessments.

TU176

How to group organotin compounds in view of their regulatory assessment under REACH?

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Organotin compounds represent a group of organometallic compounds which has attracted regulatory attention several times already. Due to a combination of known use patterns, expected/demonstrated fate and behaviour in the environment, and intrinsic hazards, this regulatory attention has not diminished – at the time of writing this abstract, the following activities have been identified under REACH: there are two restrictions (one on organotin compounds in general, and one substance-specific), four organotin substance have been included in the candidate list for inclusion in Annex XIV of REACH (authorisation list), four organotins are on the CoRAP list (Community Rolling Action Plan) for substance evaluation, harmonised classification has been proposed/agreed on for various organotin compounds, and finally, a number of other evaluative activities have been identified in the PACT (Public Activities Coordination Tool), such as hazard evaluations (PBT/vPvB assessment, assessment of endocrine disrupting properties), risk management option analyses, etc. All these regulatory/evaluative actions are now being follow up on a one-by-one basis. To come to a more efficient approach, it was considered necessary to investigate how organotin compounds may be grouped for further evaluation. Based on the currently available information in the +30 REACH registration dossiers for organotin compounds, an effort was made to map grouping and read across actions as proposed by industry. Specific attention was given to the information used to justify grouping and read across. Then, taking into account international guidance on grouping of chemicals in general and more specifically of organometallics (e.g. ECHA, OECD), as well as previous work on grouping of organotin compounds (e.g., OECD CoCAP), a framework has been proposed to evaluate stepwise whether or not an organotin compound can be included in a certain category of organotins for read across purposes for a specific (type of) endpoint. This framework indicates what information should minimally be available or be generated to allow this evaluation. Further, it is investigated to what extent information on uses can – on top of all other information – be applied to evaluate which risk management measures (if any) would be required to guarantee safe use of groups of organotin compounds. This project therefore contributes to reaching the objectives as put forward in the SVHC Roadmap 2020.

TU177

Towards the Environmental Read-Across Assessment Framework

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REACH is a Regulation of the European Union, adopted to improve the protection of human health and the environment from the risks that can be posed by chemicals, while enhancing the competitiveness of the EU chemicals industry. It also promotes alternative methods for the hazard assessment of substances in order to reduce the number of tests on animals. Grouping of substances and read-across is one of the most commonly used alternative approaches for filling data gaps in registrations submitted under the REACH Regulation. This approach uses relevant information from analogous 'source' substances to predict the properties of 'target' substances. During dossier evaluation and testing proposal examination (REACH Regulation Title VI), the justification for the grouping of substances and read-across provided by registrants is assessed by ECHA to check whether it fulfils the legal requirements. To allow transparency and consistency of the read-across assessments, ECHA published a human health read-across assessment framework in 2015 and is now developing the Environmental Read-across Assessment Framework (ENV RAAF). It aims at structuring and codifying expert judgement of complex scientific questions on the critical aspects of verifying the justification provided by the registrants. Here we present the ENV RAAF which is divided into four sequential steps. First, the assessor performs an administrative check to make sure that a meaningful scientific assessment can be carried out. Then, the provided documentation will be assessed with regards to data quality and the hypothesis or rationale will be verified. All of the information provided for the target and source substances should be considered to enable a robust verification of the hazardous properties of the target substance when comparing them to the source. When concluding the assessment, it is kept in mind that the results of the study(ies) provided for the source substance(s) are also used for classification and labelling and/or risk assessment of the target substance. Therefore, it needs to be ensured that the hazardous properties of the target substance are not underestimated. Finally, the assessment and its outcome are documented. Application of the ENV RAAF should result in a structured step-wise assessment, consistently performed between assessors, that recognises the strengths of the read-across and identifies possible shortcomings in documentation, scientific reasoning and/or supporting evidence.

TU178

Weight of evidence approach to assess the acute aquatic toxicity of GTL solvents relative to other hydrocarbon solvents

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Hydrocarbon solvents are petrochemicals used in a wide range of industrial and consumer applications globally. These products consist of many different hydrocarbon constituents and are described as UVCB substances (substances of Unknown or Variable composition, Complex reaction products or Biological materials). They are most commonly derived from the processing of crude oil, but other varieties, such as synthetic 'isoparaffins' and solvents derived by the Fischer-Tropsch, or 'Gas-to-Liquid' (GTL) process, also exist. Although different solvents may share similar performance properties, their composition varies depending on the manufacturing process and they are regarded as different substances under REACH. In order to assess the relative aquatic toxicity of GTL solvents compared with other hydrocarbon solvents a range of screening methods have been used. These included testing of water-accommodated fractions (WAFs) using solid-phase micro-extraction (SPME) combined with gas chromatography (GC) analysis, MICROTOX^{TM} and DAPHTOXKIT F^{TM} assays, and toxicity predictions using the PETROTOX model. Results were compared with compositional information for each of the substances and available experimental data from GLP compliant acute aquatic toxicity studies, conducted under OECD guidelines. The results of the screening tests suggested that acute toxicity of GTL Solvents is dependent on hydrocarbon chain length, similar to dearomatised and isoparaffinic hydrocarbon solvents. The white spirits were significantly more toxic based on screening data, which was expected based on the presence of aromatic constituents. On the basis of the experimental screening methods applied, the two most sensitive approaches for detecting toxicity of the various products are SPME-GC and the DAPHTOXKIT F^{TM} test kit, whereas the MICROTOX^{TM} assay is the least sensitive. The PETROTOX calculations predicted a similar relationship with chain length, but were generally more conservative than the experimental data. Overall, through the use of a range of screening methods in a weight of evidence approach, the acute aquatic toxicity of GTL solvents was successfully determined relative to other types of hydrocarbon solvents.

TU179

Environmental risk assessment of natural complex substances: needs for methodology adjustments

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Natural complex substances (NCS) are under the scope of European regulations on chemicals (Biocides, PPP, REACH, CLP…). For example under REACH regulation NCS are considered as substances, either mono-, multiconstituent or UVCB. In most cases however, NCS can be considered as UVCB, hydrophobic and volatile substances. The objective of the study is to identify difficulties in environmental hazard and risk assessment of these substances and propose methodology adaptations for these chemicals. Two strategies are generally envisaged for their evaluation: one considering the NCS as a "substance" as a whole or taking its "constituent" as a basis of the assessment. The approach “substance” shows the advantage to consider the effects of all the constituents of the mixture without these needing to be totally identified. For inconvenience, certain required data have no meaning for a UVCB such as the solubility, the Kow, the bioaccumulation which are parameters intrinsically appropriate to each of the constituents. Other data can be obtained by testing the substance directly. The “constituent” approach requires an important effort for the characterization of the NCS. Besides, the addition of the effects of each constituent is not necessarily equal to the effect of the mixture as such. The approach by constituent and the characterization of these allows the use of alternative methods as the approach QSAR, by congener or category and thus the read-across between constituents. Under REACH regulation, for the tonnage band greater than 10 T/year, environmental exposure and risk have to be assessed. In this case, for environmental exposure, constituents have to be taken into account, the environment acting as a filter, the UVCB as such does not exist. It can be then decided to consider the predicted environmental concentration (PEC) of the most soluble of known constituents and to compare it with the predicted no effect concentration (PNEC) of the substance. This approach can be relatively conservative and can be refined in higher tier assessment. An example of this assessment is presented to illustrate the methodology.

TU180

Data Availability of REACH Registrations: Adaptation of Information Requirements for High Tonnage Chemicals

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According to the REACH Regulation (EC) No. 1907/2006 chemicals produced, marketed or used within the European Union have to undergo a registration process, wherein the registrants have to provide information on hazards presented by the substances and assess potential risks. The standard information requirements defined in Annexes VII to X of the regulation may be waived or adapted by the registrants if adequate documentation and justification according to criteria specified in Annexes VII to XI are provided. To evaluate the availability of ecotoxicological data in registration dossiers of high tonnage substances (1000 tpa or more) and their compliance with the REACH Regulation, the Federal Institute for Risk Assessment (BfR) in cooperation with the Federal Environment Agency (UBA) developed a systematic web-based screening scheme. In total, 1932 dossiers were checked for selected environmental endpoints such as biotic and abiotic degradation, bioaccumulation and ecotoxicity. A remarkable high rate, 43% to 82 % depending on the endpoint, of the evaluated dossiers included waiving or adaptations from the standard information requirements such as read-across approaches. The assessment of the appropriateness of waiving and adaptations were part of a follow-up project. Herein, it was evaluated whether the given justifications were in accordance with the criteria set out in the respective REACH Annexes. Altogether 335 cases were evaluated for all environmental endpoints and categorized as “formally compliant” and “formally non-compliant” referring to the formal correctness, “obviously compliant” and “obviously non-compliant” indicating compliance with regards the content or “complex”. Thereby, 31 % of all cases were classified as “formally compliant” and 2 % as “obviously compliant”, whereas 12 % were assigned to “obviously non-compliant” and 19 % to “formally non-compliant”. However, 35 % were not concluded and remained as “complex”. Read-across approaches were most frequently used as justification for waiving. The results will show the frequency and pattern of waiving/adaptation approaches for the environmental endpoints. Besides this general overview, specific problems regarding the application of the REACH Regulation were identified and their relevance with regard to remaining data gaps will be discussed.

Environmental risk assessment of chemical mixtures: the steps ahead (P)

TU181

Addressing combined toxicity in the environmental risk assessment of inorganic UVCBs: some case studies

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There is a clear need to include a generic approach to address combined toxicity when assessing multi-metallic substances such as inorganic UVCBs (iUVCBs). Because the variability in composition is generally too arge, it is not possible to identify a set of samples that would be representative and conservative for the hazard identification of the iUVCB and hence experimental testing is not feasible. For this reason, the iUVCBs risk assessment is conducted based on the assumption that the toxicity is driven by the toxicity of their constituents, resulting in parallel risk assessments of the constituents. In a conservative approach, each constituent of the substance is assessed based on its maximum concentration and its most severe chemical speciation. Upon ECHA’s request, registrants of iUVCB intermediates committed to improve the the combined toxicity assessment part of their REACH registration dossiers. However, an appropriate standard approach to address the combined toxicity of inorganic constituents in a regulatory framework is still missing: most standard approaches yield indeed over-conservative results such as risk scenarios at natural background concentrations when several metals are combined. Therefore, a generic tiered approach has been recently developed for the environmental risk assessment of iUVCBs. This approach starts from the standard concentration addition evaluation based on summation of the PEC/PNEC ratios of the individual constituents and includes several options for refinement of this standard approach, e.g. taking into account bioavailability, using msPAF approaches or assessment per trophic levels. In addition, screening methods for evaluating the relative importance of data poor constituents are included in the overall approach. This poster presents the results of this tiered approach for addressing the combined toxicity in a constituents-based environmental risk assessment for some representative iUVCBs.

TU182

Toxicity and bioavailability of lubricants and their additives in the aquatic environment

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For the purpose of communicating environmental hazard via material safety data sheets and product labels, lubricants have to be classified according to their toxicity to the aquatic environment. In line with European Classification, Labeling and Packaging guidelines and GHS classification guidelines, the classification of mixtures should primarily be based upon the available test data on the mixtures as a whole or on similar mixtures. However, in those cases where these data are missing, the classification should be based on calculation (i.e. the GHS summation method) using those ingredients classified for the particular hazard. As experimental data on the aquatic toxicity of lubricants are often not available, hazard assessment of these products is regularly based upon the toxicity of the individual hazardous ingredients, the additives. Fully Formulated Lubricants (FFLs) were tested in screening tests with crustaceans (*Daphnia magna*), algae (*Selenastrum capricornutum*) and bacteria (*Vibrio fischeri*). The test results were then compared with classifications derived from applying the summation method. It is suggested that the additives might not be bioavailable to the aquatic ecosystem due to their incorporation into the lubricant (OECD, 2004). An overestimation of the aquatic toxicity of the final product may be due to a reduction in the bioavailability of the highly toxic additives as a consequence of partitioning into the lubricant base oil. To test this hypothesis, toxicity data were generated (from water accommodated fractions (WAFs)) on the additive packages containing the toxic components on their own and in the presence of base oil as the FFLs.

TU183

What do effect-based tools need to capture to support water monitoring?

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Environmental quality monitoring of water resources is challenged with providing the basis for safeguarding the environment against adverse biological effects of anthropogenic chemical contamination from diffuse and point sources. While current regulatory efforts focus on monitoring and assessing a few legacy chemicals, due to progress in analytical techniques many more anthropogenic chemicals are and will be detected in our aquatic resources. Assessing this type of exposure information based on available standard approaches from single chemical prospective risk assessment leads inevitably to indication of risk in most surface water bodies (Malaj et al. 2014, Stehle and Schulz 2015). However, exposure to neither individual chemicals nor mixtures does necessarily translate into adverse biological effects. As an alternative to generic assessment approaches effect-based monitoring approaches are suggested (Escher et al. 2014). To become a credible complement to chemical monitoring information we need, however, better understanding of the capabilities and gaps of available effect-based tools (EBT). In this work we therefore undertake to (i) compile organic contaminants detected in freshwater monitoring studies, (ii) provide a synopsis on the mode-of-action knowledge available for the detected compounds to map against available EBTs coverage, and (iii) utilise a hazard identification approach to identify priority compounds for effect-based monitoring. From our work it emerges that chemical occurrence in European freshwaters seems to be highly variable in composition and relative abundancies. Further, while we are substantially limited in our mode-of-action knowledge we can already identify major gaps in coverage of potential effect qualities when relying on established EBTs. Finally, we suggest a list of organic compounds that could serve as a reference list for EBT validation studies.

TU184

Attempt to apply the WHO/IPCS framework on assessment of combined exposure to multiple chemicals to regulatory environmental risk assessment of chemicals

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Environmental risk assessment for regulatory objectives is in general conducted on a chemical-by-chemical basis. Under the limited resources, considering mixtures assessment for all of the chemicals under the regulatory environmental risk assessment will not be effective nor practical. High-priority chemicals groups should be identified efficiently. WHO/IPCS proposed a framework adopting tiered approach for assessment of combined exposure to multiple chemicals. We have been conducting attempts to apply this framework to consider multiple chemicals in the present practices on environmental risk assessment of regulatory objectives. Concentration of chemicals in the ambient environment (air and water), which can be obtained through monitoring programs by national and local governments as well as academic studies, can provide information on combined exposure to multiple chemicals. Concerning hazard to the environment, our tentative focus is on "conventional ecotoxicity" to typical aquatic organisms. In order to examine the applicability of the framework, case studies for certain groups of chemicals have been implemented. Target groups of chemicals have been identified considering structural similarity, modes of action and co-existence in the ambient water. Updated situation of the case studies will be presented at the Annual Meeting.

TU185

FRAM - The Centre for Future Risk Assessment and Management Strategies at the University of Gothenburg

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Chemicals are typically assessed and managed one after another for their environmental risks, based on the notion that the each chemical is the only toxic substance present in an otherwise pristine environment. It is becoming increasingly clear that this strategy is a major shortcoming of even modern regulatory frameworks such as REACH, as it is a precarious oversimplification of reality, in which dozens or even hundreds of chemicals are present at any given time and location in the environment. \nFailing to account for the presence of chemical cocktails in the environment is particularly problematic because scientific evidence shows that the toxicity of a mixture usually exceeds the toxicity of each individual compound and that small, individually non-toxic concentrations might add up to a severe toxicity of the overall cocktail. Chemicals in the environment are thus a typical case of the "tragedy of the commons": even if the ecosystem impact of each individual chemical emission might be acceptable, their combined effects might not. The failure to set boundaries for the total chemical emission from multiple chemicals, sources and activities erroneously implies an open, infinite ecosystem. This obviously wrong assumption is the Achilles heel of current chemical regulation. It is not sufficient to ensure that the actions of an individual company, authority or consumer are on an acceptable level - it is their joint impact that matters for ensuring sustainable chemical governance. \nThe FRAM center is therefore set-up to provide an organizational structure for work towards defining safe local, regional and global boundaries for chemical pollution that protect ecosystem services against the impact of the totality of chemical emissions and exposures. It will develop policy instruments for the fair sharing of the available common emission space. FRAM will in particular explore how flexible and scientifically sound chemical management systems can be established in middle- and low-income countries. The center is set up at the University of Gothenburg and is run by an interdisciplinary group of environmental scientists (ecotoxicology, ecology, environmental chemistry, life cycle assessment and systems analysis), environmental economists, legal and tax experts as well as political scientists. Initial funding is provided for a duration of seven years, from 2016 to 2023.\n\n

TU186

Effect-based screening of whole blood to evaluate complex   chemical exposure of sea turtle populations in the Great Barrier Reef

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The globally endangered green turtle *Chelonia mydas* occurs in coastal and estuarine environments, where they can be exposed to a wide range of known and unknown chemicals. Target analyses are poorly suited to evaluate such exposure or associated risks. The objective of this study was to validate and test the application of bioanalytical screening approaches to investigate the combined effects of chemical mixtures in whole blood. We used green turtles from three different habitats in the Great Barrier Reef to assess whether their chemical exposure differs. Blood samples were collected from turtles foraging in nearshore areas associated with agricultural activities (Upstart Bay), industrial sources and urban runoff (Cleveland Bay), and a relatively pristine offshore site (Howicks Group of Islands). We applied a tiered bioanalytical screening approach with a battery of six *in vitro* bioassays. Baseline toxicity was tested with the bioluminescence inhibition assay (Microtox). Specific modes of action, AhR-CAFLUX and BG1 were applied to screen dioxin-like and estrogenic activities. For the reactive mode of action we used AREc32 assay targeting the oxidative stress response in the adaptive stress response pathway, NfkB-bla assay as an indicator for immune response to infection and inflammation, and p53-bla assay as a response to DNA damage. First, we tested sediment samples from the three habitats to get insights into the bioavailability of the chemicals. Then we validated a QuEChERS extraction method on whole blood samples as a non-target approach for bioassays. We showed that there were no matrix interferences in any of the assays. Furthermore, 25-30 blood samples were analysed from each habitat to test if there are differences between them. Our results showed that sediments from the offshore site (Howicks) had consistently lower responses than the two coastal habitats. For turtle blood samples, three bioassays (Microtox, AREc32, AhR-CAFLUX) were positive, with the agricultural habitat (Upstart Bay) showing significantly higher responses compared to the other two sites. Overall, this study demonstrates that cell-based bioassays provide a suitable non-target analytical tool to evaluate combined mixture effects of organic chemicals in whole blood samples. Combining such tools with information on sea turtle population health status could present a powerful tool to evaluate associations with complex chemical exposure in nearshore environments.

TU187

Is the toxicity of pesticide mixtures occurring in agricultural influenced streams additive? An experimental approach with Daphnia magna

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A broad variety of pesticides are used for crop production and may consequently be introduced during or following their application to adjacent agricultural streams. Ecological risk assessment is, however, based on single compounds. This procedure may not be protective if pesticides are present in complex mixtures due to potentially synergistic interactions. To empirically test for the protectiveness of current risk assessment approaches, we took advantage of the Swedish national monitoring program providing insights into the pesticides contamination pattern in agricultural streams. Based on these data, the acute and chronic toxicity of seven pesticide mixtures with the highest ecotoxicological potential was identified. To assess the acute toxicity of each pesticide mixture, *Daphnia magna* was exposed to the sum of toxic units ($\sum$TU), which was calculated based on the pesticides' reported individual acute toxicity, ranging from 0.0125 to 32. For the characterisation of the mixtures' chronic toxicity, daphnids were exposed to $\sum$TUs, based on the NOEC for reproduction, ranging from 0.25 to 8. It was uncovered, that a $\sum$TU of 2 to 10 is needed to cause a 50% reduction in mobility following an acute exposure. These data indicate that the pesticide mixtures show a lower toxicity than it would be expected from the pesticides' individual acute toxicity. Also during chronic exposures, a significant decrease in reproduction was observed at a ∑TU between 1 and 8, while higher ∑TU partly resulted in substantial mortality (up to 100%). The study's findings uncovered that mostly lower than additive or additive effects occur for pesticide mixtures, suggesting the concept of concentration additivity is often protective for pesticides in agricultural stream.

TU188

Does pathogen infection affect the elimination of toxic compounds?

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The importance of combined exposure to pesticides and pathogens to organism health has gained increasing recognition, mainly to improve methods for controlling pests in agricultural settings and for reducing mortality in beneficial insects. However, very few studies have quantified the interactions or described the mechanisms behind observed interactions between chemical stress and pathogen load. One suggested mechanism of interaction is *via* enzymatic processes e.g. the cytochrome P450 (CYP). CYP are initially involved in the detoxification of chemicals, and often up-regulated in response to chemical stress. On the other hand CYPs have been reported to inhibit or interfere with the innate immune response towards infections. The inverse relationship of CYP in detoxification and immune defense indicates a trade-off between these systems, a trade-off that can result in altered chemical elimination kinetics and delayed clearance of infection. The aims of this study were to investigate the effect of pathogen infection on the elimination kinetics of sublethal doses of a chemical using the flour beetle *Tenebrio molitor* as model organism. Propiconazole, a widely used azole fungicide, which has relatively low toxicity towards invertebrates, was chosen as chemical stressor, whereas the tapeworm, *Hymenolepis diminuta*, was chosen as pathogenic stressor. We hypothesized, that co-exposure to propiconazole and *H. diminuta* would result in 1) decreased CYP activity due to energy allocation towards immune defences and hence 2) decreased elimination rate of propiconazole. To investigate this, internal concentrations of radiolabeled propiconazole will be followed over time and CYP activity will be measured *in vitro* continuously before, during and after the chemical elimination phase in infected and non-infected beetles.

TU189

Spatial and temporal analysis of the risks posed by polycyclic aromatic hydrocarbon, polychlorinated biphenyl and metal contaminants in sediments in UK estuaries and coastal waters

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The environmental risks of 22 contaminants, comprising 6 metals, 10 PAHs and 6 PCB congeners occurring in UK estuaries and coastal waters were assessed as single substances. Sediment samples were taken within 12 nautical miles of the English and Welsh coastlines between 1999 and 2011. The measured environmental concentrations were compared to quality standards including ERL, ERM and EAC, all of which have been established internationally. Out of a total of 38,031 individual samples analysed, 42.6% and 7.7% exceeded the ERL/ EAC and ERM values, respectively. The highest Risk Characterisation Ratios (RCRs) for metals, PAHs and PCBs were observed for copper, fluorene and CB118 (2,3',4,4',5-pentachlorobiphenyl). In general, the highest concentrations of PAHs and PCBs were observed in 2011 in the Lower Medway indicating a potential risk to the aquatic environment. This study suggests that re-suspension of contaminants banned over 20 years ago is still an ongoing issue.

TU190

Lessons learned from testing PNECoral as reference value in a component based prediction of mixture effects of contaminants

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In the natural environment, living organisms are not only exposed to one single pollutant at a time, but to a variety of different contaminants. The exposure to a mixture of chemicals is first and foremost through food (prey), but also from water and the environment they live in. Component based-approaches are based on the concept that the effect of the mixture is a function of the effect of the individual compounds. The method of summing up PEC/PNEC or MEC/PNEC ratios, i.e. concentration addition, has been recommended as a justifiable mixture risk approximation in order to estimate in a first tier approach whether there is a potential risk for an exposed ecosystem (Backhaus and Faust 2012). Within the European regulation, the risk for wildlife and predators due to oral intake from lower trophic levels of bioaccumulative contaminants, is estimated with the use of PNECoral. PNECoral values represent dietary predicted no effect concentrations, below which food concentrations are not expected to pose a risk to birds or mammals (ECHA 2008). Results from long-term laboratory studies are strongly preferred, such as NOECs for mortality, reproduction or growth. If a chronic NOEC for both birds and mammals is available, the lower of the resulting PNECs may be used as the secondary poisoning assessment to represent all predatory organisms (ECHA, 2008). The method of summing up MEC/PNECoral ratios was used in order to evalute the potential risk for organisms in a study of urban terrestrial areas. In the study, the sum of MEC/PNECoral was calculated, where MEC was the median or 90 percentile concentration of contaminants measured in terrestrial organisms such as earthworm and bird eggs. PNECoral values were adopted from previously assessed and reported values. We will present the results from our calculations, and further evaluate and discuss the applicability of this component based approach; i.e. concentration addition for mixture risk prediction for our terrestrial urban study. References: Backhaus, T., Faust, M. (2012) Predictive environmental risk assessment of chemical mixtures: A conceptual framework. *Environ. Sci. Technol., 46*, 2564-2573. ECHA (2008) Guidance on information requirements and chemical safety assessment. Chapter R.10: Characterisation of dose (concentration)–response for environment. Helsinki, European Chemicals Agency.

TU191

Human health risk due to polycyclic aromatic hydrocarbon (PAHs) exposure in Kumasi, Ghana; and excretion of PAH metabolites in cattle urine from urban and rural sites

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Previous studies of polycyclic aromatic hydrocarbons (PAHs) in particulate matter (PM10), soils and livers of wild rats indicated that the city centre of Kumasi, Ghana was severely polluted with high cancer potency, and fuel, wood/grass combustion were the dominant sources. Cattle urine were therefore collected from Kumasi (urban) and Offinso (rural), Ghana, to determine concentrations of 13 PAH metabolites (OH-PAHs), and investigate exposure levels of cattle to PAHs from the different sites. From the results, geometric mean concentrations (adjusted by specific gravity), GM_{SG}, showed that 2-OHNaphthalene (2-OHNap) was the most abundant OH-PAH and significantly higher in Kokote (rural) than the other sites. The GM_{SG} concentration (ng/mL) of $\sum_{9\text{-OH-PAHs}}$ decreased in the order, Kokote (44 ± 10) > Oforikrom (7.8 ± 7.4) > Saboa (6.9 ± 3.8) > Santasi (6.6 ± 2.7) > and T. Estate (5.2 ± 1.5). Principal component showed high association of 2-OHNap, 2-3-OHFle, 2-OHPhe and 3-OHPhe in Kokote indicating high exposure of cattle to PAHs in this area. Studies have indicated that exposure of rats and mice to naphthalene caused nasal and bronchiolar tumours, respectively. Based on the above studies and because urine could be used as a biomarker of exposure, effect and susceptibility, we collected human urine from 3 hospitals in Kumasi, and also from KNUST campus (where PAHs in PM10, soils and livers of wild rats were significantly low) to measure the concentrations of OH-PAHs and their association with oxidative stress. Results indicated that 2-OHNap (16 ± 28 ng/mL) was most abundant and highest in participants who visited Manhyia hospital (22 ± 40 ng/mL); and exposure could be through the use of naphthalene-containing-mothballs in water purification or insecticide. Highest level of 2-OHNap, (224 ng/mL), was found in urine of a female participant in Manhyia hospital, who reported symptoms of persistent cough, headache, dizziness, tachycardia, myalgia, nasal congestion and inflammation. 1-9, 4-OH-Phes, and 1-OHPyr were most abundant from a cyesis participant who visited Manhyia hospital. GM_{SG} (ng/mL) of $\sum_{9\text{-OH-PAHs}}$ from participants in the hospitals decreased in the order, Manhyia (26 ± 41) > Tafo (17 ± 28) > Atonsu (16 ± 21). Although levels of OH-PAHs in urine collected from KNUST were significantly lower than the hospitals, 2-OHNap was most abundant. Significant correlation between 2-OHNap in cattle and human urine could indicate a common source of naphthalene exposure.

TU192

Ecological risk of mixtures of radioactive and stable chemical compounds predicted by multi-substance species sensitivity distributions

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Nuclear power plants release both radionuclides and stable chemicals to the environment under normal operating conditions, notably in river ecosystems. However, within the chemical regulatory framework, the ecological impact of those liquid releases is still evaluated based on the separate risks engendered by single substances. Until now, no attempt has considered radionuclides and stable chemicals in a holistic environmental impact assessment. This study contributes to improving existing regulatory instruments with the aim to propose an integrated risk assessment approach taking into account radioactive and stable chemical compounds altogether. Species sensitivity distributions (SSD) are widely used to estimate the potentially affected fraction of species (PAF) resulting from exposure to individual contaminant. This approach can be combined with mixture models in order to assess the multi-substance PAF (msPAF), a quantitative indicator of the ecological risk associated with mixtures. Such an integrated approach has rarely been applied to the case of mixtures that include radionuclides. Here, individual SSDs built for each radionuclide and stable chemical released to the Rhône River by 4 nuclear power plants were combined: the msPAFs associated with different release scenarios were derived using the concentration addition or response addition models according to the toxic mode of action of each substance. Due to the peculiarities of radionuclide ecotoxicology, the risk assessment is not straightforward and additional steps in the evaluation are needed. Particularly, chronic effect data are related to dose rates (energy deposited to the organisms by unit time due to ionising radiation from all radionuclides) and not directly to the radionuclide concentration in the exposure media. Those peculiarities also offer a unique opportunity to refine the 'traditional' approach (in which SSDs for each compound are derived before calculating msPAF using a mixture model) with a recently proposed approach in which mixture models are applied to each species separately before deriving a single multi-substance SSD. The latter approach is considered to be more robust, but has only been tested theoretically yet because it requires toxicity data for the same species for each compound, which is possible in the radionuclide case. The poster will compare the different approaches, and discuss their feasibility and realism regarding mixtures that include radioactive compounds.

TU193

Prospective risk assessment for mixtures of agricultural chemicals in surface water

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In March of 2015, a SETAC Pellston workshop was held to help inform decision making around aquatic mixture risk assessments of chemicals using exposure scenarios and decision trees. The efforts were broadly grouped into three areas of chemical origination: agriculture, domestic, and urban influences (a separate integration group was charged with looking at overarching issues). The agricultural land use combined effect measures with exposure scenarios of chemical mixtures for field and catchment-scale using procedures that are recognized and used in regulatory schemes in the U.S., Europe and other parts of the world. Chemicals modeled were those used in crop protection and livestock production, and were considered to occur as mixtures (in time and space). Two types of scenarios were defined including a single unit that could represent a variety of typical chemical input locations (e.g., feed lot, agricultural field, pasture, aquaculture, biosolids applications, etc). The second scenario was multi-unit, combining several different uses within a single catchment/watershed. These assessments considered inputs from spray drift, surface runoff and erosion, and/or tile drainage systems on a daily basis over an extended period of time (e.g., from one to 30 years). Case studies included a single unit scenario modeled as a wheat field in Eastern UK, consisting of crop protection applications of 13 substances over the course of the year. This scenario used standard FOCUS soil, weather and receiving water body information for consistency. The case study of a multi-unit catchment scenario consisted of a combination of corn fields, pasture, and feedlot inputs based in part on the US EPA Iowa corn scenario used in pesticide registration evaluations. Manure from treated cattle containing two different pharmaceutical substances (a parasiticide and a macrolide antibiotic) was applied to corn fields as fertilizer, and also originated from pastured cattle. Twelve different active substances for crop protection were modeled on the corn field. These applications ranged from a pre-plant herbicide to a late-year fungicide application. A risk assessment decision tree looked at estimated environmental concentrations of all active substances on a daily basis, with subsequent mixture risks evaluated according to methodologies developed under a separate effects workgroup within the same Pellston workshop. A summary of the framework, methodologies and results will be presented.

TU194

Assessing the combined effects of chemical mixtures using novel alternative tools

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Objective: Humans and wildlife can be exposed to an infinite number of different combinations of chemicals in mixtures via food, consumer products and the environment. It is practically unfeasible to test all these possible mixtures experimentally. Therefore smart strategies are needed to assess the potential hazards using new tools that rely less on *in vivo* testing and incorporate instead alternative experimental and computational tools. **Methods**: Recent literature has been reviewed and a survey on expert experiences in the assessment of combined effects been conducted. The current state of the art for the application of alternative tools was evaluated, focusing on the adverse outcome pathway (AOP) concept, *in vitro* methods, omics techniques, *in silico* approaches, toxicokinetic and dynamic energy budget (DEB) modelling, and on integrated approaches to testing and assessment (IATA). **Results**: Expert opinions in the survey regarding the use of novel tools in the risk assessment of mixtures were split between those applying them (often more in a research context) and those that generally think these tools are valuable but their use is currently limited because of lack of guidance, lack of data, or lack of expertise. The literature review showed how these tools allow deriving meaningful information on individual mixture components or whole mixtures, leading to a better understanding of the underlying mechanisms of their individual and combined effects. **Conclusion**: Using the above-mentioned tools in smart combination and an integrated way, different aspects regarding the hazard from combined exposure to multiple chemicals can be put into context. This will finally allow a better, mechanistically based prediction of mixture effects. In order to benefit from these tools in the hazard assessment of mixtures, more guidance on their use is needed to facilitate a more widespread application and integration into regulatory assessments.

Fate and Effects of Metals: Regulatory and Risk Assessment Perspective (P)

TU195

Establishing a shared methodology to develop reliable dose-response relationships for trivalent chromium

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Risk assessment (RA) strongly depends on available ecotoxicological data and requires adequate quantification of hazard (exposure-response relationships) and exposure (i.e., concentrations likely to be encountered in the environment). In the case of chromium, RA must consider the simultaneous environmental occurrence of the two main redox forms, namely Cr(III) and Cr(VI), that have markedly different environmental behaviour and reactivity toward biological surfaces. Current consensus is that Cr(VI) is much more toxic than Cr(III), so that risk assessment and management have been particularly focused on this redox form. The concern over Cr(VI) is fully justified. However, Cr(III) ecotoxicity may be largely underestimated due to the lack of appropriate consideration of Cr chemistry in standardized (and not standardized) media used in hazard assessment and in the study of Cr(III) effects on the physiology and biochemistry of living organisms. The chemistry of Cr(III) in (standardized) test media is rarely considered in detail. The main issue is that soluble Cr(III) concentrations will be controlled by the possible formation of insoluble Cr(III) hydroxides in the test medium. This is of particular concern at circumneutral pH, but can occur also at slightly acidic pH depending on the added Cr(III) concentrations and medium composition. The formation of insoluble hydroxides lowers the actual solubility of added Cr(III) and may cause a marked decrease of the Cr concentration in the test solution over test time. When these factors are not examined, exposure-responses relationship established for Cr(III) in different studies will not necessarily provide adequate information on the actual exposure concentrations. The appropriate way to establish correct exposure-response relationship for Cr(III), especially under circumneutral conditions, is to use protocols originally developed for sparingly soluble substances. Appling the recommendations of such guidelines to Cr(III) requires repeated measurements of total and filterable concentration over the entire test duration, calculation of exposure concentration as time-weighed mean or geometric mean, expression of Cr(III) toxicity in terms of (modelled) free ionic species instead of total (filterable) concentrations, and provision of apparently trivial details such as aging of the spiked exposure medium that, as for the ecotoxicity of nanomaterials, can affect Cr(III) behaviour during ecotoxicological tests.

TU196

The classification of Attapulgitic clay under CLP/GHS

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GEOHELLAS mines and processes attapulgitic clay ("fullers earth") from near-surface, lateritic deposits in northern Greece. The material is produced in various particle sizes for use as absorbents in the food and feed industry. In edible oil applications, the material is able to remove oil contaminants, including dangerous 3-MCPD. In feed applications, the material absorbs mycotoxin, reducing the need for antibiotics in animal feed. While the present material has a long history of safe use, analyses indicate the existence of constituents that could trigger CLP classification. These are: cobalt (0,02%), chromium (0,3%), and nickel (0,3%) (three common laterite components), and crystalline silica (common in all types of clays). In order to determine the material's status under CLP/GHS rules, the material was examined mineralogically and chemically (weight-of-evidence approach), and subjected to MeClas tier analysis. Potential human health hazards related to the presence of Cr and crystalline silica were ruled out using mineralogical data on respirable silica content (SWERF method) and on the valence state of Cr in the minerals present. The mineralogical data further demonstrate that Ni exists as an isomorphic substitution in the clay structure, ruling out the Carc.Cat.1 classification associated with other forms of Ni. To address potential environmental hazards, a speciation-based assessment was first done assuming that 100% of the metals are bioavailable (MeClas Tier-1). The result was an Aq. Chronic 3 classification due the combined effect of Co, Cr, and Ni. Because 100% bioavailability is not possible with the natural clay, a refinement was conducted using data from a 24h T/Dp test (pH6, 100 mg/L) on the finest fraction of the attapulgitic clay. These data indicate low release factors of < 0,1% for Co and Cr, and < 1% for Ni (percent of total metal concentrations), and allow for the release factors to be transformed into 28d-release rates (linear extrapolation; worst-case assumption). The extrapolated data give values of 2.53%, 0.59% and 23.16%, respectively. Inclusion of these release rates in the calculations (MeClas Tier-2) removed the provisional Aq.Chronic 3 classification. Based on evaluation of the hazard endpoints, it was concluded that the attapulgitic clay in all its forms should not be classified for any human health or environmental hazard under CLP or GHS.

TU197

Water Framework Directive - Overview of Water Quality Standards and initial assessment for molybdenum

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Environmental Quality Standards (EQSs) are tools used for assessing the chemical status of waterbodies. In summary, the maximum acceptable concentration and/or annual average concentration are established for priority substances. When these threshold concentrations are met in a waterbody, the chemical status of the waterbody can be described as 'good'. EQSs should protect freshwater and marine ecosystems from possible adverse effects of chemicals as well as human health via drinking water or ingestion of food originating from aquatic environments. Several different types of receptor therefore need to be considered, i.e. the pelagic and benthic communities in freshwater, brackish or saltwater ecosystems, the top predators of these ecosystems and human health. Five relevant EQS were considered for the evaluation of molybdenum: quality standard for the protection of the water column ($QS_{water,eco}$), protection of sediment organisms ($QS_{sediment}$), protection of predators (mammals, birds) from secondary poisoning ($QS_{biota,secpois}$), protection of men from secondary poisoning due to the consumption of contaminated fishery products ($QS_{biota,hh\ food}$), and protection of men from poisoning due to consumption of contaminated drinking water (QS_{dw}). The final $QS_{water,eco}$ for molybdenum in the freshwater and marine environment were 12.7 mg Mo/L and 2.28 mg Mo/L, respectively, and reflect the PNEC-values that were included in the REACH registration dossier for Mo-compounds. No formal drinking water guideline value has been adopted, but a health-based value has been put forward by WHO (2011) and is used as a starting point for the calculated $QS_{dw,hh}$ of 82 μg Mo/L, taking into account a removal efficiency of 15 %. With regard to the $QS_{biota,hh}$ for molybdenum, a value of 207 mg/kg was derived. This $QS_{biota,hh}$ must then be translated to a concentration in the water column according to the formula "$QS_{biota,hh,water} = QS_{biota,hh}$ / BAF". As the BAF for essential elements depends on the exposure concentration, there is no straightforward option for solving this equation. However, bioaccumulation data for the most sensitive organism in the aquatic environment (Regoli et al, 2012) demonstrated that molybdenum was properly regulated in aquatic organisms when exposed to concentration levels that equal the $SQ_{eco,water}$., with internal concentrations well below the critical threshold of 207 mg/kg. Therefore it was not required to derive a $QS_{biota,secpois}$ and/or $QS_{biota,hh}$.

TU198

Application of descriptive and multivariate analysis for obtaining the pollution markers of untreated wastewater in Novi Sad, Serbia

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Untreated mixed effluents (a mixture of municipal and industrial wastewater and surface runoff) are a complex, multivariate environmental systems, in which a number of physical, chemical and biological processes occur simultaneously. For the selection of an appropriate treatment process and the design of the stable conditions for optimum performance of the wastewater treatment plants (WWTPs), it's necessary to have a detailed information about the sources, composition and levels of pollutants in the wastewater. This study examined the relationship between these processes through analyzed parameters of untreated wastewater in Novi Sad, Serbia. The samples of wastewater were taken from one of the most burdened discharges in the city of Novi Sad during 2012-2014. Experimental data were analyzed by basic statistical methods for determination of mean and median values, standard deviations, minimal and maximal value of the measured parameters. Then association of cluster analysis (CA) with the principal component analysis/factor analysis (PCA/FA) was applied in order to establish a model link between parameters and sampling seasons, as well as to detect and diagnose abnormal events during monitoring periods, which helps in the design of the wastewater treatment plant in the future. Hierarchical CA grouped the eleven monitored periods into three clusters based on their similarities, corresponding to seasons of low polluted period (LP-P), moderate polluted period (MP-P) and high polluted period (HP-P). For the three different groups using PCA/FA three factor model was constructed and resulted in 70.08%, 67.54% and 76.99% of the total variance in the wastewater quality datasets of LP-P, MP-P and HP-P, respectively. PCA/FA indicates that in each period beside organic pollution primarily heavy metals are responsible for wastewater quality variation (point source: industry). These heavy metal factors are interpreted as metal pollution from the industrial effluents. Each factor has strong positive and negative loadings on Pb, TCr, Zn, Cd and Fe concentrations. Large data base obtained within this study could give relevant information about the physico-chemical status of wastewater in order to establish the best treatment technology of wastewater at selected wastewater discharge. **Acknowledgement:** This study has been financially supported by Ministry of Education, Science and Technological Development, Republic of Serbia (III46009 and TR34014).

TU199

Antimony fate modelling approach for regional nuclear risk assessment

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Antimony (Sb) is a highly toxic ubiquitous oxyanion that has poorly been studied in the environment until recently. However, there is no global view of its biogeochemical cycle and the processes taking place (behaviour, transformation and transport), especially in aquatic systems. In addition, it is a refractory fission product in nuclear power plants (NPP), released and detected after the Chernobyl (ChNPP, Ukraine 26th April 1986) and Fukushima Dai-ichi (FDNPP, Japan 11th March 2011) accidents both in soils and marine invertebrates at unpredictable sites and time lapses. The aim of this work is to determine the environmental fate and potential dispersion of radioisotopes before accidental NPP events in the Gironde Estuary (France), the largest estuary in southwest Europe subjected to two NPPs (*i.e.*, upstream and within the salinity gradient area). Measurements on stable Sb show a non-conservative behaviour along the salinity gradient with enhanced addition processes taking place during low freshwater discharges. Dissolved concentrations (Sb_d) range from ~0.12 μg l^{-1} at S=0 to ~0.4 μg l^{-1} at S>27, higher than available measurements from the Atlantic coast off Nantes (0.21 ± 0.01 μg l^{-1}). Estimated net fluxes (Boyle's method) range from ~20 to ~420 kg day^{-1}, compared to average gross fluxes of ~2.5 and ~14 kg day^{-1} registered from 2004-2014 at the estuary upstream limit (*i.e.*, La Réole). Variations in particulate concentrations (Sb_p) during different discharges along the estuary are less evident. Distribution coefficients indicate that high suspended particulate matter (SPM) favours Sb_p up to ~90% of the total Sb with SPM ~1000 mg l^{-1} (corresponding to the maximum turbidity zone, MTZ). Estimations on radionuclide generation after an accident indicate critical times for radiotoxicity exposure (*i.e.*, γ and β^- radiation) within the first 4 days (tidal scale) and 1 month after the accident, with environmental persistence of several years for the latter case (seasonal implications for the ^{125}Sb isotope: half-life of 2.76 y). This study shows that freshwater discharge and the resulting position and dynamics of the MTZ will play a major role in transport and residence times of Sb radionuclides towards the coast and within the Gironde Estuary, including the risk of upstream transport of radioactive particles to Bordeaux during summer draught.

TU200

Application of improved scientific approaches in support of metal risk assessments

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Several aspects must be accounted for in order to perform a scientifically-sound risk assessment of metals in soil, such as e.g. essentiality and variability of soil properties influencing the bioavailability and toxicity of metals. During the last 2 decades, a lot of research has been performed in these fields and numerous chronic metal toxicity data were generated for various terrestrial species and microbial functions in different soil types. From these data sets, models were developed for correction for differences in bioavailability among soils or between laboratory and field conditions. For many metals, the large amount of chronic toxicity data for different species and functions allow the application of the species sensitivity distribution approach (SSD) to derive soil quality standards. This work was mainly triggered by the European legislation on chemical management (REACH and the former Existing Substances Regulation) and the data were therefore primarily used to derive predicted no effect concentrations (PNEC) for prospective risk assessment. The data can however also be used for other purposes. For example, different combinations of effect level (EC_x) and protection level (hazardous concentration within the SSD, HC_p) can be considered for the derivation of soil quality standards. The use of the SSD approach also allows predicting the potentially affected fraction (PAF) of terrestrial organisms for a specific metal concentration in a specific soil. In order to facilitate the setting of ecological quality standards for metals in soil for different protection purposes (e.g. prospective risk assessment or remediation) and in different jurisdictions, while still making maximal use of the wealth of data and models already available, a tool is being developed that allows using the data and models originally developed for the European prospective risk assessments towards a more flexible derivation of soil toxicity thresholds. This can be done by allowing selection of the different effects level (e.g. EC_{10}, EC_{20}, EC_{30} or EC_{50}) as a basis for the assessment, the protection level required, the group of organisms to be protected, bioavailability models to be included etc. The advantages of this approach are harmonization of toxicity datasets and bioavailability corrections used and the enhanced transparency in the derivation of ecological quality standards for metals in soil for different goals and different scenarios.

TU201

Implementation of soil bioavailability concepts in the determination of German precautionary values

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According to the German Federal Soil Protection Act (1998), soil precautionary values are defined as “soil values which, if exceeded, shall normally mean there is reason that concern for a harmful soil change exists, taking geogenic or wide-spread, settlement-related pollutant concentrations into account”. In the German Soil Protection Ordinance (1999), such values are given for seven metals. Recently, more values have been proposed for arsenic and thallium. All of them were defined based on total concentrations (“aqua regia”). Regarding the pathway “soil – soil organisms” it is well known that there is a poor correlation between total metal concentrations and their effects on microbes, plants and invertebrates. There is also a general agreement that a realistic risk assessment of metals should be preferentially based on concentrations of their biologically available fraction – but it is not clear which fraction should be used for regulatory purposes and how to measure it. In any case the implementation of bioavailability should account for several aspects affecting metal toxicity to soil organisms, such as the differences between laboratory and field conditions (including ageing processes) or the variations in the properties of natural soils (i.e. pH, clay content, CEC etc.) – not to mention the form of the metal (i.e. water-soluble salts vs. metal oxides etc.). Corrections for ageing and varying soil properties have recently been used in the prospective risk assesment of metals in the European Union (e.g. in REACH dossiers). Referring to these experiences the German Federal Environmetal Agency has launched a project in order to develop a transparent concept to derive soil precautionary values considering ecotoxicological effects of bioavailable fractions of metals.. The work is divided in four steps, starting with a literature review on existing concepts on the derivation of soil values for metals. Based on the results of this compilation, a specific testing and assessment concept will be formulated as a basis for the third step, i.e. practical testing. The latter will focus on a limited number of metals, soils, ecotoxicological test methods and extraction methods. The concept will be tested for a cationic metal (copper) and an oxyanion (either arsenic or molybdenum). In the final (fourth) step all available information will be used to propose a new concept for including bioavailability into the derivation of future precautionary values.

TU202

Bioaccessibility and Health Risk Assessment of Potentially Toxic Elements in urban soils of Lagos, Nigeria

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Total or pseudo-total extraction over estimates the risk of human exposure to PTE since only a fraction is bioavailable or bioaccessible. Bioaccessibility extraction tests simulate the human gastro-intestinal tract especially of children, the target group most susceptible to accidental or deliberate ingestion of non-food substances. The protocol estimates the actual fraction that is soluble and available for absorption into the blood stream. In this work, Lagos urban soils were tested for bioaccessible PTE’s and potential risk assessment. A total of 20 soil samples were studied, comprising soils collected from public access areas and from known point sources of PTE such as dumpsites and industrial areas. Pseudototal PTE concentrations were determined by digesting 1 g of soil in 20 ml *aqua regia* using a microwave digestion system (Mars Xpress, CEM, UK). The Simplified bioacccessibility extraction test (SBET) using pH adjusted (1.5) glycine was employed. Extracts were analysed for PTE by inductively coupled plasma mass spectrometry (ICP-MS Model 7700, Agilent Technologies, UK). Copper, Pb and Zn generally showed higher bioaccessibilty (% BA with respect to pseudototal concentrations) than Cr, Fe, Mn and Ni which may reflect an anthropogenic, as opposed to geogenic origin. Risk assessment was estimated by calculating the amount of soil that a hypothetical child needs to ingest to reach a toxicologically significant level. The data were compared with tolerable daily intake (TDI) values calculated for a child weighing 10 kg using the human-toxicity maximum permissible levels published by Baars et al (2001)[1] The obtained data and corresponding daily intakes calculated indicated that all soils were able to pose risk of Pb toxicity in the event of accidental soil ingestion of 10 g/day. Only three out of all the soils exceeded TDI for Pb in the case of conservative consumption of 100 mg/day. More worrisome was one of the dumpsite soil samples in which only about 80 mg of soil needed to be ingested to deliver the TDI for a 10 kg child. The TDI for Zn was exceeded in almost half of the soils studied assuming soil pica tendencies (10 g/day). Generally, for all PTE studied, Pb appeared to be the PTE of greatest concern considering both typical and excessive soil intake by a child. 1. Baars A.J, Janssen P.J.C.M,Hesse J.M,Van Apeldoorn M.E ,Meijerink M.C.M,Verdam L, Zeilmaker,M.J, *Re-evaluation of human-toxicological maximum permissible risk levels*. Netherlands, 2001.

TU203

Introducing speciation changes, human bioaccessibility and bioavailability in the risk evaluation of dietary inorganic arsenic intake: a Belgian case study

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Arsenic (As) is an ubiquitous element frequently present in food items. Human health risks related to its dietary intake are not linked to total As intake, but depend on the chemical form (speciation) in which As is taken up. Hence normative legislation should preferably be speciation-based in the case of As. To assess the risks associated to dietary As intake, the knowledge of the internal exposure to different As-species is necessary, and this can only be determined if speciation changes during food preparation and food digestion are known. The BIOTRAs project aimed at incorporating these changes and knowledge about As-species bioaccessibility and bioavailability into the risk assessment of dietary As intake in Belgium. Different food items were analysed before and after preparation to determine the effect of preparation on As concentrations and speciation. The prepared food samples were subjected to a sequential in vitro digestion system (Unified Barge Method). To mimic colon digestion, the intestinal digests were incubated with colon suspension sampled from the SHIME reactor. The bioaccessible fraction was collected by centrifuging the small intestine- or colon digests. Apparent permeability values of As and its species from the digested food matrices were obtained by means of the Caco-2 cell system. From these data, human intestinal absorption values were derived (bioavailability). To perform As in- and uptake calculations, different food consumption scenario’s were elaborated based on the Belgian Food consumption survey and literature data. To evaluate the risk of inorganic As (iAs) in- and uptake, margins-of-exposure were calculated. An increasing consumption of typical iAs sources (e.g. rice, hijiki seaweed) increases the intake of iAs, although cooking these food items in an excess water mitigates the increase. The calculated systemic uptake of iAs for a usual Belgian consumption pattern is calculated as 7 μg/d. A high consumption of selected food items leads maximally to a doubling of the iAs intake. Ethnic groups with a rice-based diet have a 25- to 100-times higher uptake of iAs than the average Belgian population, depending on the consumed amount of rice and its preparation method. No health risks due to iAs intake or uptake are expected for the general Belgian population. A long-term ethnical rice-based diet is discouraged for people with an increased risk for lung cancer such as smokers.

TU204

Phytomanagement of eutrophic wetlands polluted by mine wastes: the role of the biogeochemical processes

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In the studies presented in this work we evaluated the effectiveness of combining liming and vegetation for the phytomanagement of saline eutrophic wetlands polluted by mine wastes. We assayed the effect of lime application and growth of the halophyte Sarcocornia fruticosa (L.) A.J. Scott on metal dynamics in pots and soil columns under variable hydric conditions. Polluted soils from the La Marina del Carmolí salt marsh with a pH~6.4 and polluted soils from Lo Poyo salt marsh with pH~3.1. Both soils showed high EC, up to 20 dSm^{-1}, calcium carbonate contents below 6 g kg−1 and low concentrations of TOC and TN. In both soils, the total heavy metal concentrations were high, especially for Al (13-16 g kg^{-1}), Fe (61-118 g kg^{-1}), Pb (5-7 g kg^{-1}) and Zn (4-500 g kg^{-1}). A lime amendment with a pH=8.9 in a 1:2.5 water suspension, derived from the marble industry, was added to each soil at a dosage of 2%, giving four treatments: neutral soil from La Marina del Carmolí with and without liming and acidic soil from the Lo Poyo salt marsh with and without liming. Pots (13.5cm×14cm) and columns (15cm x 60cm) were filled with the treatments. The experiment was performed in two phases. Phase 1: cuttings of S. fruticosa were planted in the pots, which were irrigated for 10 months with eutrophic water and soluble metal concentrations (Al, Cd, Mn, Pb, and Zn) and plant survival, plant biomass, and plant metal content were determined. Pots without plants were irrigated in the same way. Phase 2: the contents of six pots per soil treatment (three with S. fruticosa and three without plants) were transferred to the corresponding columns. Each column was placed inside a larger container filled with synthetic eutrophic water enriched in nitrogen and organic carbon. The water level (WL) in the containers was maintained at 20 cm below the soil surface for five weeks (1st High WL) and then at 45 cm during the following five weeks (1st Low WL), repeating this cycle once more along the study (2nd High WL, 2nd Low WL.The pH, Eh, and soluble metal concentrations (Cd, Cu, Fe, Mn, Pb, and Zn) were measured regularly at each depth for 18 weeks. The results of both experiments showen that choice of the most appropriate phytomanagement techniques for recovering eutrophic wetlands polluted by metal mine wastes depends on the particular characteristics of the soil-water-plant system, the hydric/flooding regime, and the type of pollutant (nitrogen, phosphorus, and metals).

TU205
Impact analysis in the frame of Life Cycle Assessment, of the toxic metals emitted by industrial activities to agricultural soil in Fez area
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Contamination by trace metals in agricultural soils in the Fez region are linked to their irrigation using water from the Oued Fez. The latter is fed by sewage from surrounding industries and the city, which have a high pollution load in spite of treatment and risk minimization's efforts. Several reports and studies in this region confirmed the state of high metallic pollution in Oued Fez's waters and its confluence with the Oued Sebou. The effluents of the tannery and textile industries as well as those of the metal finishing industry, present the greatest risk because of the use of some metals in their processes. This work aims at interpreting the metals quantities which are emitted during the industrial activities life cycle, in terms of potential impacts on water, soil and human health. The life Cycle Assessment (LCA) was adopted as multi-criteria methodology for identifying and quantifying the major contributions related to various local industries' metal emissions. In particular, it is question of analyzing the aquatic and terrestrial ecotoxicity, and human toxicity. The characterization of these impacts takes into account the fate of trace metals emissions from their sources to the waters, sediments and agricultural soils through irrigation water. Their toxic effects on aquatic species and human health are also considered. The fate of metals emissions was determined by a geochemical study in the areas at different sites. The analysis of total concentrations and bioavailable fractions was conducted on the industrial effluents and the three environmental compartments: water, sediments and soils. The effect factor is based on the effect concentrations HC_{50} of the Impact2002$^+$ database and by specifying some parameters such as the partition coefficient of trace elements, the density and the moisture content of the soil. At this stage of analysis, it could be identified that the trace elements Cr, Ni, Cu and Zn are the major contributors to the terrestrial ecotoxicity. This is due to several causes combined or separated: high total concentrations in the soil, a greater mobility of bioavailable fractions and/or lower concentrations of toxic effect.

Higher tier tests in the risk assessment of plant protection products (P)

TU206
Reflections on bird and mammal risk assessment: past, present and future
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The use of plant protection products on agricultural crops can result in exposure of birds and mammals to toxic chemicals. The risks from such exposures are assessed under the current guidance document, EFSA (2009). The risk assessment procedure is sequential, moving from worst-case screening steps through to increasingly more realistic, higher tier risk assessments. The current guidance document was designed to increase the realism of these theoretical risk assessments, in comparison to its predecessor (SANCO/4145/2000). Since its adoption over 5 years ago, many plant protection products have been registered successfully using EFSA (2009). However, there are still many cases where low risks cannot be demonstrated using the current scheme, even using extensive and robust higher tier refinements. The aim of this presentation is to discuss the implementation of the current scheme, including levels of conservatism in input parameters, and interpretation by regulatory authorities, together with proposals for how the guidance document could be improved when it is revised in the not too distant future. The content of this presentation forms the basis of an invited manuscript to be published as a 'Focus' article in Environmental Toxicology and Chemistry.

TU207
Honeybee brood studies under field conditions: is there a difference of the brood termination rate compared to semi-field studies?
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Based on EU Regulation 1107/2009/EC the current regulatory risk assessment on bees has to address the risk to honeybee larvae or honeybee brood. According to the new "EFSA Guidance Document on the risk assessment of plant protection products on bees (*Apis mellifera*, *Bombus* spp. and solitary bees)" (EFSA 2014), both, the Oomen bee brood feeding test (Oomen *et al*. 1992, EPPO Bulletin 22: 613–616) as well as the OECD Guidance Document 75 (2007) are given as the two higher tier options to refine the risk on honeybee brood if concerns are raised in tier 1. The evaluation of historical data from semi-field studies according to OECD GD 75 showed a strong variability of the brood termination rates (BTRs) as the key endpoint (Becker *et. al* 2015, Julius-Kühn-Archiv 450: 83-92). Therefore the performance of EPPO 170 field studies using the OECD GD 75 bee brood evaluation might be one option to get more reliable BTR data, which was envisaged already some years ago in 2009 (Becker *et. al* 2009, Julius-Kühn-Archiv 423: 43-44), used for several years, and followed-up by Giffard & Huart (2015, Julius-Kühn-Archiv 450: 111-120). However, broader data sets supporting the benefit of this combined methodology are still lacking. Thus, the current presentation summarises control BTRs gained under field conditions which have been performed since 2012 in Germany. They were conducted according to EPPO guideline 170 (4) (2010) with detailed brood evaluations according to OECD GD 75 and covered the assessment of one or two brood cycles during and after the exposure of the bees to flowering crops. In addition, studies covering the analysis of a 2nd brood cycle with free flying honeybee colonies subsequent to a 1st brood cycle derived from semi-field trials were also considered. Overall the evaluation covers the data of almost 50 control colonies. These data were compared to the updated findings on control BTRs from almost 70 semi-field bee brood studies (Becker *et. al* 2015, Julius-Kühn-Archiv 450: 83-92) now. Finally, the advantages and disadvantages of both test approaches are discussed.

TU208
Semi-field Study for the Honey Bee (Apis mellifera) using a Micro-Colony System
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Higher tier studies, which require the use of bee colonies and more complex test designs, are often difficult to evaluate because high levels of background mortality are seen in controls and yet the findings from these multi-disciplinary studies are used to assess the potential for risk to bee populations exposed to plant protection products. Currently, tests are frequently based the 2007 OECD Series on Testing and Assessment: Number 75 "Guidance document on the honey bee brood test under semi-field conditions," with an initial 7 day direct exposure in a tunnel after the application of the test article, followed by a 19 day observation period outside of the tunnel. This only addresses the impact on eggs present at the time of application and not the later stages of development, which some test articles are known to have an impact on. Therefore, there is a need to develop a test that is sufficiently robust to provide reliable and reproducible data within a contained environment to allow the bee colonies to survive for a period of at least 22 days (time taken for development of a newly laid egg to emergence) so that all stages of development can be assessed. This study is designed to evaluate the survival of un-treated adult bees and associated brood under test conditions to verify that control colonies exhibit adequately low mortality rates in the absence of test article to justify the use of mini-colonies of ca. 2000 adults. In addition, both conventional frames and specially divided frames, which allow the queen access to specific areas for egg laying are being evaluated. The hives are provided with adequate food stores above a queen excluder to prevent her from using the food frames for egg-laying. This is in contrast to the conventional approach of providing the minimum amounts of stores to prevent their use in preference to incoming nectar and pollen which may (in a real study situation) contain the test substance, although bees in fact only use the stores when there is no incoming food. Individual hives are manipulated to ensure that all stages from eggs to old larvae are present at Time 0 (day of application). Timed assessments of all life stages allow comparison of the previously used Oomen method with the current OECD 75 and also the traditional frames with the new divided frames. The selected hives are established in the enclosures (*ca.* 70 $m^{2)}$ of flowering *Phacelia* with the mortality of foragers being monitored during the pre-assessment period.

TU209
Minimum Detectable Differences as a criterion to assess the reliability of micro- and mesocosm studies
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The new European guidance document on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters requires reporting the minimum detectable differences for tests on significant differences between population abundances in the controls and treatments in mesocosm studies in order to assess the reliability of the statistical analysis. If the study should be used to derive a regulatory acceptable concentration under the ecological threshold option, a reliable analysis of direct effects should be possible for a least 8 potentially sensitive populations. However, no clear guidance is given

on how to assess if the MDDs are sufficiently robust. Recently, Brock et al. (2015) proposed how the MDD could be calculated and suggested also a criterion on how many sampling dates the MDD should be below specific values to allow a reliable analysis of direct effects on a taxon. Here we will present experience with the MDD concept in the re-evaluation of older mesocosm studies and the use of MDDs in studies recently conducted. We will focus on how many and which potentially sensitive taxa fulfilled the criterion proposed by Brock et al. (2015) and how this relates to different test systems, level of taxonomic identification, sampling methods, sampling frequency and mode of action of the test item. We will discuss the practicability and relevance of the MDD criterion for different taxa and the potential consequences for the role of mesocosm studies in the aquatic risk assessment in the future.

TU210

Tiered testing for sediment organisms: towards calibration of the EFSA proposal

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The EFSA Scientific Opinion (SO) on the effect assessment for pesticides on sediment organisms in edge-of-field surface water proposed a tiered effect assessment approach for sediment organisms. The aim of this study was to assess whether the tiered approach is suitable for sediment organisms. The global data requirements for sediment testing were reviewed and, example molecules covering different modes of action (insecticide, fungicide and herbicide) were selected. Datasets were compiled for each EFSA tier; i.e. laboratory, modified laboratory studies and species sensitivity distributions as well as full mesocosm studies. All data were extracted from the regulatory (EFSA conclusions, DARs, RARs and US-EPA documents) and the open literature. Based on the laboratory data Regulatory Acceptable Concentrations (RACs) in sediment are were determined. From the higher tier data, information concerning sediment-associated organisms (typically emergent insects and rooted macrophytes) is was extracted. A comparative analysis of these data is will be presented to validate/calibrate the assessment factor used to trigger additional testing (tiers 2 and 3) or conclude on acceptable risk.

TU211

Do stable isotope signatures and elemental stoichiometry mirror toxicant-induced changes in predation success?

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Contaminant-induced changes in food-web structures are difficult to detect using traditional methods employed in multi-species studies. Stable isotope signatures and elemental stoichiometry may help to overcome this problem as both can provide information about the food source utilization by consumers. To assess the suitability of these tools, we conducted an experiment with the omnivore amphipod *Gammarus fossarum*, who had the choice to feed on black alder leaves or to prey on mayfly nymphs (*Baetis* sp.). As a model stressor, we applied thiacloprid at 0.75 μg/L, which is known to increase the predation success of *Gammarus* on *Baetis*. To assess all factors potentially affecting gammarids' stable isotope signals and elemental composition, a factorial approach was employed (insecticide x *Baetis* x leaves). The consumption of *Baetis* and leaves was recorded over two weeks in microcosms (*n*=5-10) and stable isotope signals and elemental stoichiometry, for N and C, were finally analyzed in gammarids and both food sources (*n*=5). As expected, thiacloprid exposure increased the predation success of *Gammarus*. This pattern was, however, not reflected by elemental stoichiometry, neither using C, N, nor the C:N ratio. Moreover, N content differed significantly between unexposed and exposed gammarids fed only *Baetis*. As these treatments did not differ regarding predation success, this observation indicates a differential N assimilation in response to insecticide exposure, which would rule out elemental stoichiometry as a tool to trace food source utilization under chemical stress. In contrast, stable isotope signatures mirrored the increased predation success of gammarids on mayfly nymphs, while no significant difference in signatures was detected between unexposed and exposed gammarids when receiving the same single food source. Furthermore, while calculations on a mass basis revealed an increase of the proportion of *Baetis* in the diet of *Gammarus* from 15% to 20%, the isotope data showed an increase in the proportion of assimilated food from 13% to 28%, indicating a stronger effect of the insecticide on food assimilation than on food uptake. Although further experimentation is required to draw final conclusions, the present study thus indicates stable isotopes as a promising tool to mirror contaminant-induced changes in food-web structures that additionally provides information about the physiological usage of food sources being not obtainable using traditional methods.

TU212

Can we combine data from avian reproduction studies with different test species to improve risk assessments?

A. Lawrence, Cambridge Environmental Assessments / Regulatory Ecotoxicology; S. Taylor, Cambridge Environmental Assessments

When conducting risk assessments for birds and mammals potentially exposed to plant protection products, there may be more than one study available for each endpoint (acute, reproduction). The current guidance document for birds and mammals (EFSA, 2009) states that it is possible to combine acute data on different species using a geometric mean approach, and that the intended level of protection is preserved. The guidance states that for reproductive studies, the geometric mean approach is currently not supported. When more than one reproduction study is available on the same species, the guidance states that it may be possible to combine or merge the studies as if they were one study. In order to do this, various criteria must be met, including: studies conducted to a similar protocol or guideline, same key endpoints assessed, similar dose responses present in each study, same test species used, same protocol used, similar number of animals used, same endpoints measured and same test conditions used. When two avian reproduction studies are available, many of the above criteria are fulfilled, because the protocols are the same. Studies may be available for different test species, however, typically mallard duck and bobwhite quail. This poster will describe how it may be considered acceptable to combine the reproductive toxicity data for two avian test species when the endpoints are derived from very similar studies, to improve accuracy of endpoints used in risk assessment. This may be especially relevant when EC10 values cannot be reliably calculated, and dose spacing is wide, leading to large differences between NOEL and LOEL.

TU214

MDDs in micro-arthropod field testing

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Micro-arthropod field tests are carried out as part of the risk assessment of plant protection products according to Römbke et al (2009). This document provides information on experimental design, but does not give indications about the available statistical power of the test or about the effect size that can be detectable in such a field study. Recently, it has been suggested (EFSA, 2013 and Brock et al., 2015) to use the minimum detectable difference (MDD) to evaluate the statistical power of aquatic test systems and validate the usage of the study for risk assessment purposes. In order to provide a better understanding of the micro-arthropod field test we carried out a retrospective MDD analysis of a series of field studies for the order Collembola. In the publications requesting MDDs, it is stated to calculate MDDs only for species. Although in reality only the lowest taxa level that can be identified is used. The reason for the identification above species level is restrains either in time, knowledge or money. We tried to use ecological groups for Collembolans in the MDD calculation besides the taxonomical groups. The impact of grouping on the statistical power in terms of MDD will be discussed. **Brock TCM, Hammers-Wirtz M, Hommen U, et al. The minimum detectable difference (MDD) and the interpretation of treatment-related effects of pesticides in experimental ecosystems. Environmental Science and Pollution Research International. 2015;22(2):1160-1174. doi:10.1007/s11356-014-3398-2. EFSA [European Food Safety Authority] Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters. EFSA Panel on Plant Protection Products and their Residues (PPR). Parma, Italy. EFSA J. 2013;11(7):3290. Römbke, J., Schmelz, R., Knäbe, S., 2009: Field studies for the assessment of pesticides with soil mesofauna, in particular enchytraeids, mites and nematodes: Design and first results. Soil Organisms, 81: 237-264**

TU215

Arthropods in off-crop meadows in Northern and Southern Europe: More comparisons of communities and responses to insecticides

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In earlier work we analysed NTA field studies performed with the same active substance but in different cropping systems and in different regions, to provide a first insight into the importance of geographical gradient for the response of non-target arthropod communities to insecticide exposure (1). Here we present an extended meta-analysis that includes studies with other active substances and locations, but restricted to the most vulnerable habitat type (off-crop meadows, 2). Additional analytical methods were used to compare arthropod communities and their responses to insecticides.(e.g. difference in MDD's, PRC on variances). Finally, the availability and suitability of focal species or "indicator groups" (3) for use in terrestrial arthropod community risk assessment of pesticides is discussed. (1) Aldershof, S.A., Bakker F.M. (2012). Comparison of arthropod community responses to an insecticidal active in different geographic regions. In: Alix, A., et al. (2012). ESCORT 3: Linking testing and risk assessment with protection goals. Proceedings of the European Standard Characteristics Of non-target arthropod Regulatory Testing workshop held in Egmond aan Zee, 8-11 March 2010. SETAC press. (2) De Lange, H.J., Larhr, J., Brouwer, J.H.D., Faber, J.H. (2012). Review of available evidence regarding the vulnerability of off-crop non- target arthropod communities in comparison to in-crop non-target arthropod communities. External report EFSA-Q-2011-00791. http://www.efsa.europa.eu/en/supporting/pub/348e.htm. (3) Scientific Opinion addressing the state of the science on risk assessment of plant protection products for non-target arthropods. EFSA Journal 2015;13(2):3996.

Oil and Gas Extraction: Ecological Effects and Science-Based Management (P)

TU216

Effect-driven analysis for sequentially fractionated Iranian Heavy crude oil on zebrafish embryo model

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Crude oil is a complex mixture of chemicals. Therefore, identification of key toxic components in crude oil that cause toxic effects in biological receptors is a crucial, but daunting challenge. In this study, we investigated whether fractionation of oil by distillation is an effective method for investigating crude oil's toxicity via the effect-driven analysis. To this end, Iranian Heavy crude oil was fractionized into 34 fractions by true boiling point. Each fraction was separated at ten boiling point intervals. Zebrafish (*Danio rerio*) embryo screening test was employed for observing different toxicological outcomes (mortality, hatchability, time to hatch, and malformation rate), and was used to determine the toxicity of the distilled fractions of crude oil. There were no differences in time to hatch and embryo survival compare to control group, following the exposure to different fractions. However, malformation and larvae survival rates were significantly different among different fractions. Oil fractions with bulky aromatic compounds such as dibenzothiophene and phenanthrene, and their alkylated compounds, caused greater adverse effects such as severe yolk sac edema and heart edema. Our results show that identification of toxicological effects of different components of oil enable better understanding of adverse effects by specific crude oil components. (HS and JL contributed equally to this project). This work was supported by Korean Ministry of Oceans and Fisheries Project PM56951.

TU217

Assessing the Environmental Fate and Toxicity of Surfactants Used for Chemical Enhanced Oil Recovery (cEOR)

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A host of chemical enhanced oil recovery (cEOR) technologies are currently being evaluated to extend the life and maximise production of oil from new and existing reservoirs. Shell Chemicals currently produces two general classes of surfactant for enhanced oil recovery: alcohol alkoxy sulfates (ENORDET J- and A-series) and internal olefin sulfonates (ENORDET O-series). These products are currently being deployed in pilot projects around the world to demonstrate the potential of surfactant-based cEOR technology. To better understand their environmental fate and ecotoxicological effects, Shell has been commissioning a range of studies on selected products from within the ENORDET surfactant portfolio. The objective of this poster is to provide an overview of the experimental environmental fate and effects data currently available for ENORDET O-Series surfactants and how this compares with analogous classes of anionic surfactants (e.g. detergent range surfactants). The challenges faced with generating scientifically robust and relevant environmental test data for ENORDET surfactants along with potential opportunities for future research and testing will also be discussed.

TU218

Oil recovery from oil sludges obtained from different sources using surfactants in an oil sludge washing process

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Oil sludges are mixtures composed mainly of crude oil, water and sediments. These are hazardous wastes generated in the petroleum extraction and refining processes and there is a need to mitigate their impact on the environment. Treatment of oil sludges to date has been focused on physicochemical remediation and bioremediation rather than oil recovery by oil sludge washing (OSW). OSW with surfactants has been recently applied for the extraction of oil for reuse. Due to the unique nature of each sludge, this study aims to compare the oil recovery among a number of oil sludges from different sources by assessing the following parameters: surfactant type, concentration, and surfactant to oil sludge (S/OS) ratio. Oil sludge samples were obtained from oil/water separation, oil drilling, following the removal of chemical additives by heating and centrifugation, and crude oil contaminated with sediment and water. Four synthetic surfactants [Sodium dodecyl sulphate, SDS (anionic); Tween 80 (T80), Triton X-100 (TX100), and Triton X-114 (TX114) (non-ionics), and one biosurfactant (rhamnolipid, RL)] were used in this study. Surfactant absolute concentrations in terms of critical micelle concentration (CMC) were established. Cyclohexane was used as co-solvent to aid in the separation of oil in the OSW. A previous study proposed that no significant differences between toluene and cyclohexane were obtained in the oil recovery, so cyclohexane was used as an alternative co-solvent due to its lower hazard. Recovered oil was measured gravimetrically. OSW parameters effects on the oil recovery rate (ORR, %) were matrix dependent ($p < 0.01$). RL and SDS had higher oil recovery rates compared with other surfactants for all sludges. In general, high oil recovery rates were obtained at a high S/OS ratio (5:1). There were no differences among surfactant concentrations ($p = 0.745$), and the highest oil recovery was obtained at 5 CMC. Recovered oil was composed mostly of C_{10}-C_{18} aliphatic fractions, and it is suitable to be reused as feedstock for heavy fuel oil production. In conclusion, RL can be used as a surfactant to recover oil from oil sludges since they have an equivalent capacity to synthetic surfactants along with a lower toxicity, additionally low concentrations can be used to reduce costs.

TU219

Adapting chemical risk assessment for water systems related to unconventional hydrocarbons

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Chemical risk assessment is typically done per compound with a focus on human and environmental effects. Assessing the chemical risk of unconventional drillings on water quality presents difficulties that require adaptions to this risk assessment process. The scope of this literature review is to identify and describe uncertainties and knowledge gaps of chemical risk assessment relative to unconventional drillings, and adapt the process accordingly, where possible. An extensive literature study was aimed at answering the following questions: (1) How to assess a large number of chemicals, and how complete are the databases available for physico-chemical properties and toxicological information?; (2) How applicable are available models for environmental fate modeling related to unconventional gas and oil activities?; (3) How applicable are current water quality monitoring methods to unconventional drillings? More than 7500 different chemicals are used during unconventional drillings. Advanced mass spectrometric techniques, such as liquid chromatography coupled with hybrid linear ion trap (LTQ) FT Orbitrap mass spectrometry, complemented by effect-directed analysis is the best option for broad screening of such a large list of candidates. There is however a lack of comprehensive physico-chemical and toxicological information available for assessing the screened chemicals. Moreover, available environmental fate models do not consider changing chemical behavior under high temperature and high pressure conditions. In addition, there is a need for more field-based approaches for the development of groundwater models. Current water quality monitoring is typically done at the surface and in the shallow underground, and thus does not take into account the potential for deep underground leakages. Underground leak probabilities are therefore not known. Baseline data and long-term monitoring are generally also not ensured. The tools and information necessary for chemical risk assessment of unconventional drillings on water quality are not all available. There is a need for further research into physico-chemical and toxicological information, chemical behavior under downhole conditions, and more field-based groundwater models. Baseline, long-term and deep underground monitoring would allow for a more comprehensive assessment.

TU220

Development of a Generic Exposure Scenario (GES) under the EU REACH Regulation, for substances used in high-volume hydraulic fracturing operations in response to EC Recommendation (2014/70/EU)

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The European Commission Recommendation (2014/70/EU) on minimum principles for high-volume hydraulic fracturing includes, in Section 10, the requirement that substances should refer to "hydraulic fracturing" in their REACH registration dossier if they are to be used in such applications. In response to the recommendation a team of industry representatives was established that includes members from the International Association of Oil & Gas Producers (IOGP), the European Chemical Industry Council (Cefic) and the European Oilfield Specialty Chemical Association (EOSCA). The team have been working on developing a Specific Environmental Release Category (SpERC) that is based on realistic data and assumptions for hydraulic fracturing as an intended use. It can then be used in REACH dossiers as part of a Generic Exposure Scenario (GES) that describes the necessary operational conditions (OCs) and risk management measures (RMMs) which should be implemented to control the risks to the environment associated with substances used in hydraulic fracturing applications. The SpERC was developed in tandem with a background document, which provides extended information on the operational conditions, available monitoring data and assumptions that would be implicit in the final SpERC. This poster presents the SpERC that has been developed for use by industry as part of a Generic Exposure Scenario for substances used in hydraulic fracturing fluid additive products, and the conditions that are specified in the SpERC to ensure safe use. In addition,

some of the assumptions made and the details of these assumptions as presented in the background document are also reviewed.

TU221

Hot spots identification, sediment management and environmental risk assessment within a gas production field thorough a multidisciplinary approach and a novel expert decision support system

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Offshore production of oil and gas involves some of the most advanced and massive engineering projects. Such industrial activities may induce both occasional as well as long lasting environmental impacts in the aquatic ecosystem, often acting in combination with other environmental stressors. Nowadays, complex and advanced multidisciplinary monitoring approaches combining chemical, ecotoxicological and ecological data are available worldwide. These collect large dataset of different information, which need to be correctly inferred and integrated to provide a comprehensive knowledge of the environmental health status. Indeed, a management focused on impacts of a single stressor is inefficient and often ineffective because co-occurring human activities lead to multiple simultaneous impacts on communities and individual species. Thus, a quantitative assessment of the spatial patterns of all human uses of the marine aquatic environment and their cumulative effects is needed for implementing the ecosystem-based management, the ecosystem services conservation and the sustainable exploitation of natural resources processes. In this case study, we apply methods developed to map cumulative impacts in sediments collected during the key steps of the set up and the production phases of a gas platform located in the Adriatic sea. Sampling sites are classified by means of comparing chemical concentrations with effect-based target values. The level of each contaminant and the combined toxic pressure are used to rank sites into uncontaminated, mildly and heavily contaminated. According to a triad based approach, in contaminated sediments, an environmental risk index is determined by means of integrating chemical data with ecotoxicological and ecological parameters. Furthermore, a sediment risk index is computed from combining chemical and ecotoxicological data. At sites identified as moderately contaminated, sub lethal stress index are integrated with chemical data into a biological vulnerability index. In the meantime, potential risk for human health is assessed in selected stations by integrating genotoxicity biomarkers. Finally, geostatistical tools are applied to show the space and time related risk index distribution.

Development, standardization and implementation of LCA and integration with economics for transportation infrastructure and operations (P)

TU222

Noise impact category implementation in an LCA software tool to assess road restorations

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Several difficulties have arisen in the inclusion of road traffic noise as an impact category within the LCA method. There are many reasons for this marginal treatment of noise in LCA. Main reasons are related to the special characteristics of noise as a pollutant, such as the site-dependency of impacts, the human perception issues or the non-linearity of impacts and limited availability of data, which have hindered its inclusion within this method. Nevertheless, some specific studies within the LCA framework aimed to better accommodate the assessment of its impacts have appeared so far. Most of them have been focused not just in traffic noise but also in noise from any other source, from Müller-Wenk (in the 90s) to more recent authors, such as Franco, Cucurachi, Moliner or Althaus (in the last 10 years). This study presents how the endpoint methods of Moliner and Franco,considering the human health impact of noise could be implemented in an LCA software tool, named Air.e LCA®. This proposal has been developed under the framework of a new Spanish R+D+I project whose main aim is to research and develop new materials and techniques to improve the road restoration activities. The fact of including the noise impact assessment will contribute to compute the benefits to human health of developing and using new sound-dampening, sound-reduction or anti-noise materials into road surface.

TU223

Environmental assessment of EAF steel slag use in road

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Electric arc furnace (EAF) slag is a non-metallic by-product of steel production by electric arc furnace process. About 12 million tons of steel slag are produced annually in Europe. Considered as waste for a long time, this industrial by-product is increasingly used in civil engineering applications as its valorization allows a number of benefits such as the conservation of natural resources, the reduction of waste storage volumes, the decrease of material construction cost and transport demands and the promotion of local economy. The environmental assessment of EAF slag recycling using life cycle assessment (LCA) in road is investigated in this paper. Generally, in the LCA framework, the environmental assessment of recycling waste don't takes into account the phases of life cycle corresponding to stockpiling and use, as they are considered to have no impact, only waste processing is counted. Thus, we propose a method to assess recycling of EAF slag in road considering the phases of production of EAF salg (for recycling purposes), stockpiling (lixiviation test), transport (30 km) and use in road (uncovered 10-15 cm thick road layer) and to compare this scenario to natural sand in order to evaluate the various impacts obtained for each step of the EAF slag life cycle and discuss the interest of various solutions considering both toxicity and ecotoxicity indicators. Furthermore, the calculation for use phase based on experimental data (results from percolation test) is compared to its calculation based on Ecoinvent data which take into account the total content (x-ray fluorescence) and a transfert factor (from Ecoinvent data base) for short term, in order to seek if the results are in the same range or not.

TU224

Life-cycle GHG emissions of alternatives for collecting and transporting municipal solid waste: The case of a Spanish community

D. Garrain, CIEMAT / Energy Energy Systems Analysis Unit; I. Herrera, Y. Lechon, CIEMAT / Energy Dpt   Energy Systems Analysis Unit

Currently, the communities and municipalities are increasingly aware of managing a clean and sustainable transport in their mobility services. Thus, it is usual (and almost mandatory) to include aspects for improving sustainability in tenders for contracts and execution of public services. More specifically, in the case of collecting and transporting municipal solid waste several key aspects are important to be considered in order to reduce emissions, energy consumption and other environmental issues. In this context, both the use of alternative fuels and the introduction of electric vehicles are positively valued in order to achieve environmental improvements and energy savings. This study presents an assessment of different proposals for introducing the use of alternative fuels (such as liquefied petroleum gas, natural gas and biofuels instead of conventional diesel) in the vehicle fleet of a Spanish small urban community to collect and transport municipal solid waste. The possible use of hybrid and electric vehicles has also been considered. The results show an important decrease in GHG emissions when natural gas is used as fuel. Nevertheless, this measure entails an increase of other harmful gases, such as CO or VOCs, when compared to other alternatives. Finally, in order to complete the environmental assessment of the whole activities regarding the management of municipal waste, the quantification of annual GHG emissions of the processes of washing containers and pneumatic collection of waste have also been included. These activities could be responsible for the 10 to 40% of total GHG emissions of the waste management in the community.

TU225

External costs of electrification of road transport: approach and setting-dependency

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The use of electric vehicles in comparison to internal combustion vehicles is a typical example for burden shifting from the use phase to upstream processes, i.e. fuel supply in the form of electricity. Within the collaborative EC project SCelecTRA (2012-2015), funded within the frame of the Electromobility+ Initiative, the following steps in the assessment chain were addressed: 1) definition of scenarios to enhance electromobility uptake in Europe in the mid to long term; 2) modelling their impact on the power and transport sector with the aid of the TIMES PET36 model, distinguishing 36 European countries, different means of power production and different transport demands; 3) assessing the environmental implications particularly in terms of emissions and abiotic resource uses following LCA principles, and 4) monetary valuation mainly of impacts due to classical air pollutant emissions (external costs). The study undertook to establish a set of new regionally specific unit damage cost factors (UDF) for the characterisation of human health impacts in LCIA including weighting that aim at being setting-dependent, as suggested by the expert panel of the Basel workshop (Fantke et al., 2015). To this end, pre-calculated external cost factors have been obtained from the literature. For power generation, a distinction is made between different countries and emission heights (above or below 100 m or undetermined). For transport-related emissions, external cost factors specific to countries and/or setting (e.g. population density) are used to the extent available. While relying on LCI data and energy system analysis results from project partners, external costs for the different scenarios were quantified. Comparing the overall UDFs obtained this way with those derived generically for the EU27 shows a variability of about a factor of 0.2 up to 2. The use of country-specific characterisation factors (UDF) instead of EU average values for a European wide assessment of energy and

transport activities thus leads only to a negligible difference in the results. The relevance of using country-specific or generic UDF also depends on the scenario analysed.

TU226

Building a common base for LCA benchmarking of Li-Ion traction batteries

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Although the environmental impacts of e-mobility are usually dominated by the use phase, the production of the battery also plays a significant role, especially when it comes to choosing the most adequate battery type for a given application. Numerous studies quantify the impact of the battery production process along the life cycle for different battery chemistries. Nevertheless, these studies use different impact assessment methods and different approaches for modelling key parameters like energy demand for cell manufacturing or electricity mixes. On the other hand, the outcomes of these studies are highly sensitive on these parameters, why a direct comparison of different studies is critical. A common basis for comparing the environmental performance of different alternative Li-Ion battery chemistries is not given, but seems very desirable to support decisions of technology developers. Based on a review of all available LCA studies on Li-Ion batteries, the discrepancies in the key parameters between these studies are identified and their impact on the outcomes of the studies pointed out. The existing primary inventory data (LCI) for the principle Li-Ion battery chemistries are then recompiled and average values used for these parameters. In this way, the environmental impacts associated with the production of different battery chemistries are assessed on a common base. This provides an improved comparability between studies and allows for a technology benchmarking of different Li-Ion battery chemistries. Furthermore, a sensitivity analysis is done by varying the values of the key parameters within the identified range, pointing out the relevance of each of the parameters and the uncertainty in this regard. It can be observed that different assumptions made for these parameters can overrun the differences between battery chemistries completely. Especially the approach for modelling the cell manufacturing energy demand influences the results significantly. Thus, putting existing LCA studies on a common base is essential for battery technology benchmarking and avoids erroneous conclusions when comparing different Li-Ion battery chemistries regarding the impacts associated with their production and with their use in electric vehicles.

TU227

Regionalized life cycle impacts of worldwide coal transportation from mines to power plants

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Motivation Coal-fired power production is known to substantially contribute to global health problems and environmental damages. Driven by consumer demands, these are caused by pollutant emissions occurring along its entire supply chain including transportation. Due to growing world-wide energy consumption, this technology remains essential for reliable energy supply at competitive costs in most countries around the world. Thus incremental improvement of the coal supply chain is a promising opportunity for mitigating harmful impacts beyond improvement of energy efficiencies of power plants. One key problem for allocation of resources to these improvements is insufficient assessment of the regional resolution of emissions and impacts. These differ substantially depending on the transporting vehicles and environmental conditions. As such, species-rich habitats or densely populated regions are often particularly sensitive to transportation emissions compared to remote locations. This aspect has not been covered sufficiently by current publications yet. The present work aims at closing this gap by combining various types of regionalized information and calculating region-specific impacts. *Methods* Power-plant-specific consumption data is merged with global coal transportation routes to identify hotspots of the coal supply chain. Impacts on humans and ecosystems are quantified with spatially-explicit characterization factors by regionalized life cycle assessment (LCA). The required data for power plant coal demands is calculated from the WEPP database which is linked to international coal trade via multi-regional input-output (MRIO) data from Exiobase. This in turn is connected to coal mining data with regional coal specifics and explicit mine locations to determine transportation profiles and distances for different types of coal. *Results and conclusions* The results highlight hot spots in global coal transportation and show where modernization of vehicle fleets and restructuring of material flows is most relevant. As such the results are a step towards a fully regionalized inventory of the global energy sector and represent a guiding example for expanding regionalized life cycle assessments to transportation systems.

TU228

Evaluation of innovative products and processes for road infrastructure projects towards the LCE4ROADS Certification System methodology.

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Although there are many initiatives in the market capable to assess sustainability aspects for roads, many lack of a holistic view of sustainability because some of the current approaches do not cover all life cycle phases or all sustainability pillars and focus mainly on environmental aspects. Some examples are Greenroads, Envision and Invest, in the US, and Ceequal (UK), and the Dutch CO2 Performance Ladder in Europe. The purpose of this paper is to show the results achieved on the development of a new sustainability certification system for roads named "LCE4ROADS", currently under way as part of the FP7 project "Life Cycle Engineering approach to develop a novel EU-harmonized sustainability certification system for cost-effective, safer and greener road infrastructures (GA No 605748)". LCE4ROADS is based on a Life Cycle Engineering (LCE) approach: all the aspects of sustainability (Environmental, Economic, Social, and Technical) are considered with the final goal of creating a holistic and EU-harmonized methodology for assessing the sustainability of both new and rehabilitation/maintenance road projects, works and products. The proposed certification system relies on current EN and ISO standards and considers previous developments from other research projects like MIRAVEC, EVITA, COST 354 among others. Key aspects at European level such as resilience to Climate Change and the implementation on TEN-T corridors are also considered within the certification system. This paper aims also to show the first results of using the LCE4ROADS Methodology for evaluating different innovative products and processes for road infrastructure projects. This is line with one of the main purposes of LCE4ROADS certification system which is to provide support and guidance to relevant industry stakeholders on the selection and implementation of technologies that could enhance sustainability of road projects. The results show that there is difficulty in achieving a positive result on the environmental domain without negatively influencing the others, which supports the assumption that real sustainability actions should be based on trade-offs among all the sustainability domains. In conclusion, the LCE4ROADS methodology, together with its associated software tool and guide, becomes a solid method for assessing road projects and products in terms of sustainability as well as for supporting future procurement and GPP processes in Europe.

TU229

Life cycle assessment and cost benefit analysis for carbon capture and storage

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Recently, a variety of mitigation and adaptation measures on climate change have attracted attention. In particular, CCS are growing interests from year to year. According to the roadmap shown by the International Energy Agency (IEA), many of mitigation procedures have been evaluated compare their priority. Many studies, evaluating environmental performance for CCS using LCA has been performed. However, most of studies focus on only GHG emission. On the other hands, there are few studies which apply the comparison and verification including the other types of environmental impact and cost analysis. Considering the above point, in this study, it was conducted an exhaustive evaluation including environmental influences such as SOx and NOx. Further, we examined the usefulness of mitigation measures and the relationship of trade-off of between environmental impacts by calculating the total cost results including the external costs.

TU230

Environmental impact assessment of rail freight intermodality. Energy related emissions

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BRAIN-TRAINS is a project supported by the Belgian Federal Government that deals with the possible development of rail freight intermodality in Belgium, approaching the problem from an interdisciplinary perspective. Life Cycle Assessment (LCA) methodology will be used to analyse the sustainability impact of rail freight intermodality for three divergent Belgian scenarios by 2030. This objective is directly linked to the goal of increasing the rail market share by 2030, stated by European Commission's White Paper on transport (2011). The LCA rail freight system is divided in three sub-systems: rail transport operation, rail infrastructure and rail equipment (locomotives and wagons). The life cycle phases of construction, maintenance and disposal of rail infrastructure and manufacturing, maintenance and disposal of rail equipment are analysed. The sub-system rail transport operation includes direct and indirect processes that are connected with the train activity. As direct processes, we consider the specific energy consumption per travelled distance, the amount of transported freight expressed per tkm (both depending on the train traction, thus considering diesel and electricity traction separately), the direct emissions to air related to the diesel combustion in locomotives per tkm and to soil from braking and lining (iron abrasion of rail tracks, wheels, brakes and overhead contact lines) and the people exposed to noise due to rail freight transport activity. Indirect processes include the upstream emissions and energy consumption related to the production and distribution of the diesel and electricity used in rail transport will also be determined. Appropriate emission factors will be calculated to determine the direct and indirect emissions. The transport emissions related to the energy consumption during the rail transport activity will be determined considering the predicted transport volumes in a time horizon 2030.

TU231

The model for optimal transport selection based on multi-criteria decision making and life cycle assessment

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Considerable amounts of resources are used by transportation vehicles throughout their whole life cycle - from design and production, over maintenance and operation to the end of life stage. When considering transport selection problem, nowadays environmentally conscious companies are paying special attention to environmental burdens where life cycle analysis (LCA) presents valuable assessment tool. Selection of optimal transport alternative for both - employees and goods, that considers the life cycle environmental burdens is a multi-criteria decision making (MCDM) problem. This paper proposes the model for optimal transport selection based on MCDM where the environmental aspects are considered through the life cycle perspective. An illustrative example has been provided for the verification of the proposed model. The transportation problem evaluated here is of a company that needs small packages to be delivered from point A to point B in urban environments. Proposed MCDM & LCA model considers environmental, economic, social and technical aspects for selection of optimal transport expressed through defined criteria. Environmental criteria is expressed through LCA of delivery vehicle considering the following processes: production, operation, maintenance and end of life. Economic criteria is presented through the travelling cost, as social criteria the personal subjective judgement is included, while in technical the delivery time criteria (expressed through the period of time needed for package delivery) is the most important one. The considered alternatives include: transport by foot, bicycle, scooter, car, and public transportation (bus and tram). The aim of the proposed MCDM & LCA model is to assist companies which activities are occasionally connected to just-in-time delivery business, in their environmental impact decrease.

TU232

Systematic LCA method in automotive sector

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Life Cycle Analysis (LCA) is often viewed by companies as the most relevant tool to open the dialogue with stakeholders (Schmidt 2010). But on the other hand, it is percieved as a time-consuming, difficult to implement, with short-lasting relevance of its results and, above all, costly approach. The requirement of critical review for comparative LCA to communicate results to the public ,often leading to additional delays and costs, can also discourage to adopt this approach. In parallel companies are facing more and more demanding environmental regulations and standards such as the Euro regulation in the transport sector, as well as regulations that require robust communication on the environmental performance of marketed products. For example, the ongoing Product Environmenal Footprint (PEF) experimentation should lead to new regulations on environmental footprint communication based on LCA approach, that are expected in 2018. In that context, large companies have integrated environmental criteria (regulatory requirements as well as internal objectives) in their core business quality approach leading to the definition of very detailed processes that are automated/computerized in order to be easily repeated for any product. Thus, the group Renault endeavours to carry on on this environmental integration into the quality approach and to develop a systematic LCA method with the following objectives of (1) communicating on the environmental footprint of their product in an more efficient, rapid and robust manner, (2) ensuring the quality and reliability of data (notably regarding geographical, technological and time representativeness), (3) being able to improve constantly the approach and the modelling. The development of the systematic LCA method in Renault started in 2011 when Renault renewed its calculation model during the study of an innovative product, the electric vehicle. This first step of the model was assessed by a particularly skilled panel which cumulate 50 years of experience (Morel 2014) and set the baseline for further studies. The second step of the model developed in 2014 aims at comparing any new vehicle with its former model. The current model enables to perform a comparative study in about 15 days, with a critical review performed in 2,5 days on a 1,5 month period. The extended abstract described the developed method. The oral presentation with two speakers, one of Renault and one of Solinnen present the method from each point of view.

TU233

Life cycle assessment of underground construction Tunnel case study

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In a global context of urban density, the underground space can be an opportunity for developing further cities with restricted spaces, and respecting at the same time the requirements of sustainability which lie strongly today to urban projects. The underground developing in urban zones provides an important space which is characterized by its ability to offer new spaces, separate functions, protect from negative impacts, in addition to many environmental and social benefits. In parallel, the construction and the exploitation of the underground facilities can significantly affect the environment, but can otherwise provide energy efficiency due to the thermal isolation or to the energy recovery. In return, these facilities can represent a significant financial investment, generally determined in the decision-making process. This article aim is to: I) propose a characterization methodology of underground structures depending on theirs geometry, functionality and method of construction. II) Propose a methodological approach for the evaluation of their environmental impacts. This approach is based on a case study. It is a linear underground structure; that serves multimodal displacement (light vehicles, public transport, cycling and pedestrians). This evaluation is performed by environmental impacts which are obtained from life cycle assessment (LCA): energy consumption, global warming, photochemical ozone creation potentials, toxicity, and acidification. The results serve also to compare the performances of this structure to those of alternative surface solution, ensuring the same service. The question of impact's allocation to users of this multimodal transport infrastructure is also envisaged, taking into account the multimodal displacement in our case study. The results of allocation allow demonstrating the importance of mode-phase of life cycle to evaluate the environmental profitability, and then evaluate other sustainable development indicators, that will bring to think about indicators that provide results to enhance the benefits of underground construction. This initial work fits with a thesis IFSTTAR-CETU PhD, supported by the national project "*VILLE 10D-VILLE D'IDEES www.ville10d.fr*" about the design and the planning of the underground spaces to develop sustainable cities.

Expanding LCA: looking at organizations and at new policies (P)

TU234

EXPERIENCES IN THE APPLICATION OF ORGANIZATIONAL LIFE CYCLE ASSESSMENT IN MEXICO

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The evaluation of the environmental performance has been a strategy for setting goals related with the continuous improvement in organizations, but these evaluations are usually partial, because normally considered a specific facility and a reduced period. The Organizational Life Cycle Assessment (O-LCA) permits the determination of environmental indicators scientifically stronger because they consider all products and/or services of an organization, including the inputs and outputs throughout the life cycle of all associated processes. Taking into account the above, this paper presents the experiences in the implementation of the O-LCA methodology in the Central Bank of Mexico. The scope of this study concerns the banknotes factory whose function is the production of all denominations of Mexican bills. The system boundary includes all departments that are part of the factory (direct activities), the steps related to the supply of raw materials (upstream activities) and the steps related to the distribution, use and end of life (down-stream activities). For the definition of the reporting unit it took into account the production in 2013 with all related information. The assessment of environmental impacts was conducted with ReCiPe method and Ecoinvent 3.1 database, using Umberto 7.1 software. The impact categories analyzed were: agricultural land ocupation, climate change, freshwater eutrophication, human toxicity, ozone layer depletion, photo-oxidant formation, acidification and water use. The results show that for all the categories analyzed, activities upstream and down-stream together are generating the highest environmental impacts. The analysis of the direct activities shows that the departments of printing, distribution and administrative services presents the highest environmental impacts. It can be concluded that transportation, in direct and indirect activities, generates significant impacts, therefore improvements are recommended in this sector. The application of this methodology gives as a result a lot of potential improvements throughout all stages of life cycle. Scoping is an issue that can be discussed from other experiences in the application of O-LCA, since in this case the departments with the most significant environmental impacts were considered and not the organization as a whole, due to the rigorous level of detail and the large amount of data required in this type of LCA.

TU235

The Organisation Environmental Footprint (OEF) pilot on copper production

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In the context of the "Building the Single Market for Green Products" initiative of the European Commission (COM 2013 196 final and related Recommendation 2013/179/EU), which proposes a set of actions to overcome confusion of consumers due to the stream of environmental information they receive, two Organisation Environmental Footprint (OEF) pilots are currently ongoing (copper sector and retail) and they are expected to deliver the final OEF sector rules (OEFSR) by end 2016. The OEF pilot on copper production is led by the Joint Research Centre (JRC) and it is currently supported by leading European industries in the sector (Aurubis, KGHM and Outotec). Copper is a key enabler of resource efficiency, as it enables the recovery of several different products that can be associated to its production. Copper smelters can process both primary and secondary raw materials, therefore they are both at the beginning and at the end of the life cycle of products. The product portfolio of a copper producing company covers many products, all directly linked to copper production: copper cathodes, anodes and blister, sulphuric acid, iron silicate, anode slime, $NiSO_4$, $CuSO_4$, silver, gold, PGM concentrates, lead, Pb-Sn alloys, tin, crude selenium and tellurium. An organisational LCA, compared to a product LCA, considers the organizational activities as a whole: this approach allows to identify what are the most important environmental issues both in the sector and the organisations itself. It is useful for an organisation to rely on a robust measurement tool, such as the OEF, which can be implemented in other environmental management schemes (e.g. EMAS), to allow prioritisation of environmental management actions, both within the organisational boundaries (i.e. direct activities) and outside (i.e. upstream and downstream, indirect activities). The focus, therefore, is mainly on performance tracking and improvement. Results can be communicated depending on the target audience, and they can be used to provide information to investors, stakeholders and public administration. The first draft of the OEFSR on copper production was approved in July 2015 by the Environmental Footprint-Steering Committee. The ongoing work within the pilot and the final OEFSR will show opportunities and limitations of the methodology.

TU236

Evaluation of Natural Capitals for organizations Considering Life Cycle of Perspectives

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In recent years, environmental evaluation of the organizations using the framework of LCA is now paid attention internationally. Although the interests in CSR and triple bottom line increase, the development of assessment method covering 3 aspects (environmental, economy and society) of organization is still limited. On the other hand, international organizations such as the World Bank and the United Nations discuss about framework of sustainability and indicators of subcategories. These outputs would be useful to apply for the development of LCA for organization considering all aspects of sustainability. In this study, we develop a research framework of organizational Life Cycle Assessment using Genuine Saving proposed by the World Bank. Genuine Saving is a method which evaluate increased or decreased of capitals. Genuine Saving can be obtained by the summation of savings, education payment the loss of natural capital including forest, minerals, and fossil fuels and environmental damages caused by global warming. We adopted Input-Output analysis and Life Cycle Impact Assessment to evaluate these above elements and aggregate them to consider the all attributes of sustainability. We discussed the feasibility to construct a framework of Organization LCA proposed by this research based on the case studies for Japanese companies.

TU237

RECO-INNO: An evaluation model approach for eco-innovation and its impact in the business strategy

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The scarcity of natural resources and the consequences of environmental challenges affect companies that are looking for more efficient processes and sustainable products. These factors strengthen the consolidation of the eco-industry and the implementation of Eco-innovation.\n Eco-innovation improves the technology upgrading, products and services linking the binomial firm-innovation and cost savings. It is related to business models based on a new strategy to incorporate sustainable development in business, reducing environmental impacts and saving raw materials through innovation initiatives. During this last decade, the European Union has led to the improvement of environmental and competitive position of European industry with new management models and the latest technological developments. With nearly 200 million Euros, it has established the objective of promoting eco-innovation within its Framework Program for Competitiveness and Innovation. The aim of this study is to define the company's code of conduct in a sample of eco-innovative companies through the development of models based on quantitative methods. Principal determinants of eco-innovation throughout their organizational life cycle are analyzed for integration of structural variables and they are applied to the appraisal of principal economic, social and environmental outputs in business. In order to carry out the study, a sample of 100 Spanish companies has been analyzed. Data have been obtained through a questionnaire in which variables have been identified to reflect the decision about eco-innovation and its principal determinants (technological options, investment, structural and environmental variables). The keystones of the business strategy related to eco-innovation have been analyzed as well with principal business results and the company's environmental management factors. The results gained reflect an in-depth analysis of the situation of the level of penetration in Spain of eco-innovation among companies, in order to overcome principal barriers.

TU238

How sustainable are WEEE? A review of LCA applied to waste from electric and electronic equipment

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The increased use of electric and electronic equipment (EEE) has turned their waste into a pollution problem worldwide. As a complex environmental burden, over the years the management of waste from electric and electronic equipment (WEEE) has been the object of a number of Life Cycle Assessment (LCA) studies. For this presentation, we reviewed 47 LCA publications evaluating end-of-life alternatives for WEEE. Our initial point of interest were recycling technologies, but was soon expanded to include the whole waste management: collection and transport, alternative treatments—landfill and incineration—as well as waste prevention strategies. The reviewed publications focus more on products than on processes, being more interested in the impacts of a given waste flow than on a given treatment technology. Most of them take into account the whole waste management—usually considered as transport and treatment, but studies evaluating only recycling technologies are also common. Publications where recycling is confronted with other treatments are less abundant and there is even fewer taking into account waste prevention strategies, either on their own or as an alternative to treatment. We only took into account studies that covered the whole life cycle if they had a particular focus on waste management. Those that did, concluded that waste management was not the most polluting stage. The environmental importance of waste management--namely of recycling—was due to the potential recovery of valuable materials—metals, plastics, and glass in particular—could avoid the extraction and processing of new materials. However, recycling is not necessarily the greenest alternative. Long-distance transport can make landfilling more attractive and there are several waste prevention schemes that, at least in the short term, could be more beneficial to the environment than recycling. Because of that, when conducting an LCA on WEEE management strategies we should take into account more scenarios than an alternative recycling technology.

TU239

State of the art on coupling input-output table with material flow analysis in order to evaluate environmental impact of metals in an economic system

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In a context of increasing **global demand** of metals (e.g., for building and high technologies), European countries, especially France, widely depend on foreign imports. This situation implies a real risk of physical or economic disruption of supplies. The European commission registered critical materials in a list for which data concerning flows should be improved and reliable. However, it is complex to calculate material balance of a substance at a geographical scale (including import, export, waste, stocks, recycling). Improving the knowledge in material flows in an economic system would contribute to evaluate the potential of secondary raw material sources.Currently, two main methods are used to map the flows of metals: **Input-Output Tables** (IOT) which is a top/down approach and **Material Flows Analysis** (MFA) which is a bottom/up approach. The **IOT** represent the exchanges in monetary value allocated to different economic sectors. These values are contained in a square matrix showing the monetary exchanges of materials between the activities. After conversion from price flows to physical flows, we obtain physical input-output tables (PIOT). Nevertheless, the stocks and waste flows cannot be reliably calculated with these tables. On the other hand, the aim of **MFA** is to quantify the whole flows (inputs, outputs, stocks) of a substance (or a product) at a process scale with defined boundaries. However, we do not necessarily know the flows for each process, and missing values would generate inconsistencies. A reliable "mapping" of metal flows from production to final use could be obtained by coupling these two methods. Eventually, life cycle assessment (LCA) can provide **environmental impact** regarding MFA results in order to obtain a multi-criteria analysis, which depends not only on resources flows. We provide a first attempt for coupling PIOT and MFA in order to evaluate **copper flows** in France.However, such an approach deal with various data from statistic studies, custom services, professionals or scientific research. These data can be restricted for some materials.It is needed to reconcile the data from PIOT and MFA for specific materials. A methodological challenge is to develop an **innovative approach** for improving the coupling of these two methods and to reduce the uncertainties of initial data. From the resulting flow mapping, we will be able to calculate more effectively environmental impact of metals.

TU240

Eco-design of packaging for a circular economy in the food canned industry

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Since the industrial revolution, waste has constantly grown. This is because our economies have been dominated by a one-way or linear model of production and consumption that follows a "take-make-consume and dispose" pattern of growth in which goods are manufactured from raw materials, sold, used and then discarded or incinerated as waste. [1]. For this reason, in 2014, the European commission (EC) published a communication on the circular economy which stimulates innovation in recycling and reuse, limit landfill, reduce losses of resources and create incentives for behavioural change, turning waste into a resource as part of "closing the loop" in circular economy systems [2]. In the packaging sector, the circular economy model is about more than just recycling. It handles all stages of product´s lifecycle, namely: its design, production, distribution and use, but also its recovery. Regarding the product design, eco-design takes the environment into account reducing the product´s negative impact on the environment throughout its lifecycle while maintaining the same level of quality [3]. In this context, this work aims to apply the concepts of circular economy and eco-design under a life cycle approach to the seafood sector and, in particular, to the canned anchovy industry. This sector generates a high amount of packaging wastes of several sizes (0.05, 0.5 and 1 kg) and materials (glass, aluminium, tin, paper, cardboard, plastics, etc.). The life cycle assessment (LCA) of the production of one can of anchovy from cradle to gate revealed that the manufacture of these packages has the highest environmental impacts and, thus recycling these materials would improve the environmental performance of the system. For that reason, this work proposes a methodology that combines the circular economy and eco-design concepts to reduce the environmental impacts of food canned industry. [1]. Ellen MacArthurt Foundation and McKlnsey & Company. 2014. Towards the Circular Economy: Accelerating the scale-up across global supply chains. World Economic Forum. [2]. European Commission. 2014. Towards a circular economy: A zero waste programme for Europe. Brussels. 25 September. European Commission. [3]. Conseil National de l´emballage. 2014. Packaging and circular economy: a case study of the circular economy model. French Packaging Council.

TU241

LCA of Rare Earth Magnet Recovery in the REMANENCE project. The role of LCA in the development of recovery technologies; allowing circular economy of WEEE.

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REMANENCE is an ambitious project designed to dramatically increase the amount of rare earth materials recovered and remanufactured from existing waste streams, which contribute to the technology development necessary to make possible circular economy across EU. Co financed by the Seventh Framework Programme, the project brings together European industry and academia across the supply chain to develop innovative technologies, business models and market information required to exploit this valuable resource and reduce dependence on primary sources. Within this context, new and innovative processes are being demonstrated for the recovery and recycling of rare earth (RE) containing neodymium iron boron magnets (NdFeB) from a range of Waste of Electrical and Electronic Equipment (WEEE), and resulting in the development of new recovery technology able to recover RE material in a form that could easily re-enter the primary magnet manufacturing production process, so providing large energy savings and material costs. REMANENCEwill provide a secondary source of materials for the EU, large enough to supply the entire EU bonded magnet manufacturing industry and a significant proportion of the EU's high value sintered magnet production (1500-2000Tpa). Among others environmental indicators, it is expected than the introduction of the RE magnetic recovered and recycled material into the manufacturing process will significantly reduce the energy cost and environmental impact of these replaced materials. Thus, the recovery and reprocessing of RE magnetic materials is foreseen to reduce energy consumption by more than 95% when compared to emissions from primary production (mining, separation, purification and manufacturing) of RE. The analysis of such environmental indicators is possible through the use of Life Cycle Assessment (LCA) methodology, which allows comparison between the environmental impacts of the recovery technologies developed in the project versus the conventional extraction of virgin material, two very different ways of obtaining the RE material necessary in the primary magnets manufacturing. The LCA results will demonstrate how the REMANENCE technology is contributing to close the loop and boost business related to WEEE.

TU242

Recycling Rare Earth Elements

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Many rare earth elements (REEs) are used in clean technologies such as wind turbines and in permanent magnets found in electric vehicles. Today, the recycling rate for rare earth elements is less than 1%. It is expected that in the coming years, demand for rare earths will grow as the European economy makes the transition toward high-tech and green products. However, as the virgin REEs market is monopolized by China making REE prices highly volatile, and as the environmental impact of REE mining is large, the recovery of the REEs is extremely important for both economic and environmental reasons. As highlighted by the European Rare Earths Competency Network (ERECON), recycling of rare-earth magnets should receive top priority. The following study will provide summary of the current recycling techniques discovered for REEs in permanent magnets. This analysis is executed by means of a literature review and interviews with experts from both industry and academia. In addition, the performances of current recycling methods are evaluated from both an environmental and economic perspective. For this evaluation data was gathered through numerous Life Cycle Assesment (LCA) and Life Cycle Costing (LCC) experiments on REEs in permanent magnets. Neodymium is the most common rare earth element found in permanent magnets and will be assessed to determine where the greatest environmental impacts, or hot spots, in the recycling routes occur. Different impact factors are calculated, enabling detection of potential new recycling routes. Since permanent magnets contain a high concentration of REEs, a reduction in the demand for virgin sources has the potential to shift Europe's reliance on Chinese exports, as well as lowering the environmental impact generated.

TU243

Consequential Life-Cycle-Assessment as policy-support tool to promote green concretes

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The manufacturing of Ordinary Portland Cement (OPC), normally used as a binder in concrete production, is responsible for 5% of global carbon emissions. Potential sustainable alternatives, namely "green concretes", are currently studied in scientific literature. In green concretes, OPC is replaced with by-products coming from other industries. At present, however, the economic and environmental consequences of the introduction of green concretes into the market are not clear yet. This paper aims at filling this gap by using a Consequential Life Cycle Assessment (C-LCA) for the case of green concrete made from Stainless Steel Slag (SSS), with a focus on the Flemish market (in Belgium) for construction blocks. For the present study, a green concrete block was produced using SSS as OPC replacement. Today, SSS is considered as an hazardous waste, therefore stabilization is needed before its recycling as low-quality aggregate. Alternatively, SSS can be also chemically activated to produce a new binder for green concrete, hence foregoing the need of SSS stabilization. Therefore, the potential switch in the recycling of SSS, from low quality aggregates to green concrete, affects both the construction blocks and the low quality aggregates markets. In order to analyse the environmental consequences of these changes in both markets, a C-LCA was performed including all processes that are significantly affected by the changes, namely the production of traditional concrete, the production of natural/secondary low quality aggregates and their transports. In order to understand how mechanisms of revenue maximization and price equilibrium are affected by the changes, market information and economic models are also considered during the Life Cycle Inventory phase. Finally, the recycling of SSS as a binder for building materials will reduce the emission of CO_2 and NO_x , thanks to the avoided use of OPC. At the same time the change in the final use of SSS causes an increase use of natural aggregates for low value applications, unless alternatives from other by-products are considered. The C-LCA approach indicates that replacing a material by a more sustainable option does not lead necessarily to a better overall sustainability. Policy makers should therefore consider interactions between different markets when analysing the effectiveness of policy-support actions, like the increase of the tax on the CO_2 emissions or subsidies for the use of SSS as a binder.

TU244

LCA for supporting a sustainable transition towards circular economy

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Circular economy is an economic model based on resource optimization while fostering of the use of waste as resource. Within more circular economies, resource are used efficiently within the life cycle of a products and the waste generated along the supply chains are minimized and used as much as possible, directly of after transformation, as input for other products and systems. Supporting circular economy means improving the analysis of resource flows within sectors and products, defining and implementing new ecodesign strategies, ensuring low-toxicity in products and waste, as well as fostering waste prevention and recovery. Moving toward more circular economy requires that different waste management options are systematically scrutinized to assess the environmental impacts and benefit associated to them. The present work aims at illustrating how a sensitivity analysis could be applied to the impact assessment step supporting the interpretation of a LCA study applied to a waste management system that includes material recovering. The focus is on toxicity-related and resource-related potential impacts as they are considered among the most critical ones, which may affect the way the final benefit from material recovery is evaluated. Possible alternatives in terms of impact assessment assumptions and modelling are tested by performing a sensitivity analysis on a case study on electric and electronic waste. For the toxicity-related impact categories, a sensitivity analysis is performed focusing on the role of metal toxicity and long term emissions in upstream processes. For resource related impact category, a sensitivity analysis has been performed adopting different sets of characterization factors based on existing models for minerals and metals as well as recently proposed sets accounting to critical raw materials. The application of LCA is crucial for assessing avoided impacts and uncovers potential impacts due to material recovery. However, contrasting results may stem from the application of different assumptions and models for characterization. A robust interpretation of the results should be based on systematic assessment of the differences highlighted by the sensitivity, as guidance for delving into further analysis of the drivers of impacts and / or to steer eco-innovation to reduce those impacts.

TU245

Life cycle assessment of co-digestion on a wastewater treatment plant

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The increase of electricity self supply is a aim on municipal wastewater treatment plants (WWTP). A significant share of energy demands on WWTP is provided by biogas, generated by anaerobic digestion of sewage sludge. The biogas can be utilized in combined heat and power plants (CHP). Digestion of co-substrates is applied to increase the biogas yield and thereby energy self supply. However, an increased substrate input into the digester comes with an higher energy demand on the WWTP; moreover, an alternative usage of the co-substrate is hindered. Consequently, changing energy flows within the system have to be assessed. For assessing the trade-offs between an increase of energy self supply on the WWTP and additional energy/substance demands as well as the competing use of food residues as a substrate for electricity generation and for fertiliser substitution, Life Cycle Assessment (LCA) is used. A parameterised LCA model is built to assess different quantities of food residues fed into the digestion process on the WWTP. The model allows assessing plant-internal changes of energy and material demands/flows depending on different feeding amounts of co-substrates and the consequences coming from the substituted alternative treatment of food residues. Data for material and energy demands for this case study comes from a WWTP with a population equivalent of 240,000 in Germany. In the analysed case study, an additional amount of co-substrate (food residues) of about 15 weight percent is necessary to provide energy self-sufficiency on the WWTP. Due to the high energy content and the fast degradability of the organic matter of food residues, the increase in electricity self supply on the WWTP overcompensates additional energy and working material demands for treatment. Considering an alternative treatment of food residues, the total system-wide environmental impacts mostly depend on the CHP efficiencies on the WWTP / alternative biogas plant. Electricity self sufficiency on WWTP can be achieved by feeding a relatively low amount of substrates with a high energy content and using energy on-site. Depending on efficiencies of the digestion and the CHP plant on the WWTP and the competing biogas plant, co-digestion is not necessarily environmentally beneficial from a life cycle perspective. It is therefore necessary to include different treatment options and process efficiencies to assess impacts across systems.

TU246

Plastic waste treatment strategy in Switzerland: LCA approach based on parameterisation for robust decision support

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In this study we looked at post consumer plastic waste treatment options such as material recycling (open loop and closed loop if feasible) and thermal utilisation (plastic is not allowed in landfills in Switzerland). This study aimed at the strategic question: How should plastic waste from households be collected and utilized in Switzerland to lower the environmental (and economic) impact? A more specific question was: Does it makes more sense to continue the separate collection of specific plastic products like PET bottles or is there a higher benefit collecting a larger amount of plastic by doing a mix plastic collection like in other European countries. The main focus was on creating an utmost parameterized model using the SimaPro software v8.02. The variation of parameters such as transportation, sorting efficiencies, recovery rates, material quality, efficiency of energy recovery in the municipal waste incineration, electricity and heat mix substitution allowed a holistic scenario analysis for optimisation potentials regarding collection processes, recycling processes and thermal utilisation processes. The findings of the model analysis showed that the existing separate PET-collection is meaningful but the present system could be improved by expanded separate-mixed collection like PE-bottles and beverage cartons or PE-bottles and films . The actual Swiss recycling system represents a scenario which is rated as average in a sense of functionality but it does still leave room for improvement compared to the best options resulting in reduction potentials of about 20 % in the next 15 years. Interestingly, the mixture of all plastics (mixed collection) performs worst mainly due to the negative influence to the material quality and sorting efficiency. In summary, an improved recycling system for the whole of Switzerland would be ecologically worthwhile and would allow an improvement and expansion in the plastic recycling system. The model proofed to be useful for evaluating different options regarding measures taken in the field of circular economy of plastics.

Methodological challenges for LCA of agricultural supply chains producing food, fibre and bioenergy (P)

TU247

Methodological challenges for LCA of agricultural supply chains producing food, fibre and bioenergy (poster)

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LCA theory and practice still feature a number of methodological challenges, some of which are not fully solved by the research community. When LCA is applied to agriculture (i.e. cradle to farm gate), as well as to agri-food, bioenergy and fibre systems (i.e. cradle to processing plant gate) and supply chains, specific challenges arise, regarding for instance, modelling of direct emissions, allocation strategies and their consequences, land use and land use change (LULUC) and their impacts on biodiversity and climate change, responsible sourcing of agricultural products, the role of LCA to assess production and utilisation of bioenergy; as well as the extrapolation of farm level assessment to describe agricultural regions. Among methodological challenges, models for direct emissions in particular have been continuously developed and expanded to address the transportation and fate of pesticides (see FATE track), LULUC, carbon sequestration, and specific soil and water emissions, among other topics. A key question regarding direct emission models would be whether a common methodological framework is possible and desirable. Regarding the use of LCA to compare competing agricultural strategies, including bioenergy and fibre production, both at the farm, regional and supply chain (i.e. beyond regional) levels; certain methodological challenges hinder the validity of these comparisons. This session intends to advance discussion on methodological challenges to agricultural LCA and its solutions, with emphasis on convergence of methods towards a common framework acceptable for both academia and industry. It will appeal to LCA practitioners studying agri-food, bioenergy and fibre-based systems and supply chains, but also to modellers dealing with agricultural systems at the field, farm and regional levels. The presentation of case studies is acceptable when it contributes to introduce methodological developments towards overcoming current (and future) challenges of LCA of agricultural systems and supply chains, including bioenergy production systems.

TU248

Challenges for improving iLUC negative effects of biofuel demand in Spain

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From a consequential approach, biofuel life cycle greenhouse gases (GHG) analyses could present great differences if the effects of the indirect Land Use Changes (iLUC) are considered. These potential iLUC impacts relate to the unintended consequence of liberating more carbon emissions due to land-use changes around the world induced by the expansion of croplands for biofuel production in response to the increased global demand. The Energy Systems Analysis unit of CIEMAT has carried out some previous studies in order to analyse the iLUC effects, in terms of CO_2 emissions, of biofuel consumption in Spain. Results showed a great interval of variation of figures for the majority of biofuel types, especially in sunflower and rapeseed biodiesel, with average emissions ranging from 20 to 60 g CO_2 eq / MJ in biodiesel, and from 16 to 60 g CO_2 eq / MJ in bioethanol. The main reason for these large intervals was due to the selection of the location of raw materials. This study is divided in two parts. First part presents an analysis of the main economic and biophysical key factors for reducing negative effects from iLUC, such as the high dependency of the elasticity of prices, the choices of types and geographical location of crops, or the consequential use of coproducts from biofuel production. Finally, an example of the implementation of some of these measures in previous studies of iLUC effects of biofuel demand in Spain is presented.

TU249

Environmental Impact of Food Losses from Agriculture to Consumption in Switzerland

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Twenty to thirty percent of the environmental impact of consumption are caused by food consumption (Tukker et al., 2006). A key element to make our food system more efficient and sustainable is the reduction of food losses across the entire food value chain (Quested and Johnson, 2009). However, for the implementation of measures against food losses it is important to know which losses are environmentally most relevant. We quantified the amount of food losses at the various levels of the Swiss food value chain (agricultural production, postharvest handling and trade, processing, food service industry, retail, and households) in terms of mass and energy (Beretta et al., 2012). About one third of the food available for Swiss consumption (production and imports) is lost in terms of metabolisable energy. However, the environmental impact of food losses do not only depend on the amount of food loss, but also on the type of food, the degree of processing, the level in the food chain on which the losses occur, and the method of treatment (incineration, composting, anaerobic digestion, feeding). Therefore, we quantified the environmental impact of food losses at the various stages of the food value chain in Switzerland. Based on the mass and energy flow analysis, twenty-three food categories are modelled separately, representing the whole food basket. For the impact assessment the categories climate change, ecological scarcity 2013, water, and land use are considered. The results show that the food losses at the end of the food value chain cause the highest environmental impacts. Firstly, they are quantitatively relevant, representing roughly half of the overall food losses; and secondly, they cause higher impacts per kg of product because of the accumulation of the impacts of the previous stages of the food chain. The net environmental credits from the treatment of food losses are between 5 and 10% of the impacts of the supply chain allocated to the losses. Therefore, avoiding food waste is much more effective than optimizing the method of treatment. About half of the environmental impacts from total avoidable food losses can potentially be avoided by solely addressing households. Furthermore, the highest environmental benefits from avoiding one kg of food loss of average composition can be achieved in households and food service institutions. This can help public and private decision makers prioritize their strategies for preventing food losses.

TU250

Country-based and spatially-explicit Land Use Change matrix

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LCA of bio-based products present some specific methodological challenges. Among those, modelling Land Use and Land Use Changes (LULUC) and forestry (LULUCF) has proven particularly controversial. Despite recent developments, neither remote sensing nor country-based land use statistics can alone provide spatially-explicit information on LULUC by land category. Satellite images cannot always distinguish between managed and unmanaged land (e.g. forest) and shows limits in determining percent tree cover for low cover density; national statistics present limitation in terms of data quality and consistency between countries. In order to overcome these limitations, we combined the strengths of the state-of the-art remote sensing land cover datasets *Collection 5 MODIS Land Cover Type* and the *FAOSTAT* and *Forest Resources Assessment* (FRA) *2015* statistical databases. The aim was to obtain a country-based land use change matrix, as suggested by the IPCC Good Practices Guidance for LULUCF, where the area undergoing a transition between all possible land use categories between two reference years is represented by grid cells geographically identified.\n\nThe outcome was the first country-based consistent set of spatially-explicit LUC matrices. The matrices allow advanced LUC analyses aiming at: identifying: where natural land is converted and into what land type; where is cropland expanding and where is decreasing; identify land not in use or unproductive arable land; the location and therefore the potential productivity of land in transmission etc. Four versions of the matrix were generated per country, representing different levels of land-use-categories aggregation, suitable for different type of analysis. Country LUC matrices were also generated for three reference years: 2001-2012, 2005-2010 and 2010-2012 based on consistent available data and a draft version for 2010-2015. \n\nThis study represents the first attempt to consistently generate top-down LUC matrices for all countries. The matrices identify the area undergoing a transition between all possible land-use categories represented for two reference years. The methodology generating the LUC matrices used existing available data to obtain a spatially-explicit representation of land transformation. These LUC matrices intend to be a starting point for further country-specific detailed analyses, based on on-site data sampling.

TU251

Modelling the environmental effects of selected agricultural management strategies with regional statistically-based LCA

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Despite the farm being considered by many as the most suitable level of decision making and strategic management in agriculture, there is an increasing interest in evaluating agricultural management strategies at the regional level. Recent initiatives attempted to aggregate and generalise farm-level life cycle inventory (LCI) data and life cycle impact assessment (LCIA) results to describe the environmental performance of agricultural regions. We propose a regional statistics-based approach for constructing virtual representative farms (VRFs), representing dominant farm types for a given region, as a tool for comparing alternative regional agricultural strategies in contexts of insufficient farm (e.g. LCI) data. The proposed method has been applied to the agricultural Brittany region, in France. The environmental impacts of different agricultural management strategies were estimated at the regional level, by modelling the strategies as changes in VRF-based LCIs, calculating LCIAs, and extrapolating their means to the total land use in the region. Based on this assessment, performed using a regional life cycle assessment (LCA) framework, we have analysed the relative environmental impacts of each management strategy on the region. By adding a concise qualitative economic assessment, a strategy comparison matrix was built to allow farmers and other decision makers to understand the implications of implementing each strategy. The method aims to approximate regional environmental impacts from agriculture, including farm-level changes in management practices propagated to the regional scale. This work is currently under development.

TU252

Combining Life Cycle Assessment and economic modelling to assess effects of agricultural policies on the environmental impacts of the French dairy sector

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European dairy production is facing great changes. Removal of milk quotas, increased farm size and increased demand for dairy products are the drivers for these changes that are expected to affect the volume of raw milk produced, the structure of the milk production sector and the environmental impacts of the milk produced. Following the Common Agricultural Policy Health Check in 2008, impacts of quotas removal on prices and milk supply in the European Union (EU) and/or its Members States (MS) have been widely studied. However, supply and environmental impacts of this policy change through its effects on milk production systems and their share in EU and/or MS milk production, have been less studied, and, to our knowledge, never through a Consequential Life Cycle Assessment (CLCA) approach. We combined LCA and economic modelling to assess environmental impacts of public policies in the French dairy sector through CLCA. MATSIM-LUCA, a partial equilibrium model, was used to project agricultural markets to 2030, based on OECD forecasts. Several scenarios, representing different policy and food demand contexts, were simulated: i) SRef, which is the baseline scenario, representing the agricultural markets in 2030; ii) $SRef_0$, representing the agricultural markets in 2030 if dairy quotas had still been in force; iii) $S1_{LD}$, corresponding to SRef with reduced growth of world demand for milk as compared to OECD forecasts; iv) $S1_{HD}$, where SRef is implemented with increased growth of world demand. For each scenario, MATSIM-LUCA gives results, for the various regions considered (including France and EU) for: i) the share of dairy production systems; ii) the quantity of milk produced, consumed and traded, the prices for milk and agricultural goods; iii) the areas for crop and grasslands. These outputs are used to feed a LCA model. CLCA, through scenario comparison, is then performed. A range of impacts are examined, including land use change and intensification of crop production.

TU253

Developing a data-driven model to quantify farm-specific greenhouse gas life cycle emissions for open-field tomato production on a global scale

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Greenhouse gas life cycle (GHG-LC) emissions from crop cultivation consist of biogenic carbon emissions from land use change, nitrous oxide (N_2O) emissions from fertilizer application and fossil emissions due to use of machinery and material inputs. Biogenic greenhouse gas emissions vary spatially as a consequence of variation in soil composition and phytomass organic carbon as well as differences in farm management practices and technologies (e.g. tillage). Furthermore, N_2O emissions are directly related to the amount of fertilizer applied. Variability in fossil emissions is primarily caused by technological differences across different farms in different regions while variability in yield is amongst others attributed to temporal climatic differences, e.g. temperature and rainfall. There has so far not been a study that considers spatial, technological and temporal variability of GHG-LC emissions of crop production at a high spatial resolution. The objective of this research is to construct a data-driven model to estimate farm-specific GHG-LC emissions per kg of tomato produced. For this, data of open-field tomato cultivation from multiple farmers were collected in 16 countries. Within each country, data were available for a number of farms for 1, 2 or 3 years. Data include, among others, locations of farms, local climate, farm yield, soil quality including soil organic matter, pesticide application, fertiliser input, energy consumption, and water consumption.

TU254

Water use as a regional LCA indicator in the production of bio-based succinic acid using corn and wheat as potential primary resources in France

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Water availability and usage is of increasing concern in modern society (Vink & Davies, 2015). In LCA practice has been pointed out that water use is an indicator that should be assessed in the local or regional context, (Pfister, et al., 2009; Vink & Davies, 2015; others) so the use of regional data is more relevant to obtain more reliable values for this indicator. Bioeconomy is currently targeted as a strategic axis in the European Commission country members, in France particularly, it is expected to support the depletion of non-renewable resources and it is aimed to achieve a sustainable production model of biomass sources (Colloque Bioeconomie France, 2015). Bioeconomy growth could imply putting more pressure on water availability, depending on local conditions, thus it is important to review the increasing biomass uses regarding its implicit water use as an LCA indicator at regional level. As part of the bioeconomy, several biobased chemicals like biopolymers or chemical building blocks are increasing and they are expected to increase their production (NNFCC, 2015). The present work aims to contribute on sharing the experience to calculate water use as an LCA indicator, using regional inventory data for the production of wheat and corn in the regions Picardie and Alsace in France, assuming that these crops are the primary raw materials for succinic acid production in the country. Regional Life Cycle Inventories for crop production were obtained through an organization having access to specific data in the selected regions. Intermediate life stage inventories were obtained from literature, as well as from Agribalyse French database as a third inventory source for crop production, to observe differences when using average national data. Methodologies used to estimate water use are ReciPe 2008 and ILCD with adaptations to include water scarcity factors (as presented by A.M. Boulay et al., 2011). Discussion and results cover the life cycle water use in the set scenarios, and the methodological issues to estimate the LCA indicator.

TU255

The challenge of combined assessment of environmental impact and supply of essential fatty acids in omnivore and vegetarian diets

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The relationship between food and environment has been gaining attention in recent years, as a consequence, some life cycle assessment studies have taken into account not only energy or protein intake but also more sophisticated nutritional quality indicators. We used LCA methodology to assess four contrasting diets consisting of foods usually consumed in France. We investigated an Average diet, a Healthy diet, a Healthy diet without fish and a Healthy vegetarian diet, where the Healthy diets correspond to recommendations regarding macronutrients in France. Research has shown that increasing omega-3 fatty acid content of animal feed may result in higher omega-3 fatty acid content of animal products. Several non-fish products with higher omega-3 levels are available in the market in France. For each of the four diets we investigated the environmental impacts of the substitution of standard animal products (milk and beef, sheep milk, goat milk, rabbit meat, chicken, egg and pork), wheat flour and oil by the corresponding omega-3 enriched food items. To assess the environmental impacts the following impact categories were used: global warming (GWP) acidification (AC), eutrophication (EU), land occupation (LO), cumulative energy demand (CED) and biotic natural resources depletion-species (BNR-eco). Moving from Standard to increased omega-3 versions of the four diets improved nutritional quality without increasing environmental impacts. Shifting from Average diet to Healthy diet improved nutritional quality and decreased environmental impacts by 10 to 30 % depending on the impact. Shifting from Average to Vegetarian diet reduced environmental impacts by 11% to 49%, but decreased nutritional quality in terms of omega-3 contents. These results highlight the challenges of carrying out LCA studies of diets and stress the importance of the inclusion of nutritional quality indicators which go beyond kcal intake or macronutrient ratios. This work was supported by the French AGRALID project (ANR-12-ALID-0003)

TU256

Impacts of food consumption: a missing hot spot in LCA?

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Use stage (i.e. food consumption) impacts are inconsistently accounted for within LCA and likely constitute a hot spot of considerable magnitude with respect to human health impacts of food systems. As case study examples, methods are lacking to assess global and regional nutritional impacts and to account for exposure to chemicals within food contact materials (e.g. packaging). This study's objectives are to develop methodologies to address *consumption* of food products and associated impacts as a potential hot spot within LCA. We extend life cycle thinking (LCT) to a broader disciplinary context to raise awareness in the LCA community about global and regional public health "hot spots", e.g. identified through epidemiology. Data is collected to build an inventory of chemicals within food packaging materials, and we estimated and characterized exposure, and identified major research gaps and areas of prioritisation. Also to investigate consumption as a potential hot spot, regional and global epidemiology-based models were developed to estimate disability adjusted life years (DALYs) associated with various dietary unit-mass intakes (e.g. gram of sodium). Applying life cycle thinking (LCT) and a broader disciplinary context can help redirect environmental sustainability assessments towards priority regional and global human health issues specifically with respect to food systems.

TU257

Joint assessment of environmental Life Cycle Assessment results and grape quality using multicriteria evaluation with fuzzy logic

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Life Cycle Assessment (LCA) addresses different environmental impacts so that it provides a set of indicators results that can be contradictory. Deciding as well as comparing systems on more than one criteria becomes rapidely an impossible task. This becomes even more complicated when the number of indicators is increased by broadening the assessement to other issue like product quality.The objective of this work is to agregate a large number of LCA results which could be compared with the product quality assessment of a vine plot. LCA results can be aggregated by means of a tool called CONTRA (TRAnsparent CONstruction of decision trees). CONTRA is a method using fuzzy inference system to avoid threshold effects in aggregation. This tool aggregate several indicators into a single quantitative score. The advantage of such a method, when applied to LCA, is thus to study the LCA of a system through a single result in order to compare it to a single score obtained in the same way from product quality assessment. The comparison of environmental and quality issues is then simplified to the comparison of two scores. The first step consisted in defining with experts the decision tree: selection of criteria and their organization. Then, the evaluation model was constructed by defining the CONTRA parameters according to experts' opinion: definition of threshold values for each criteria, criteria weighting, and generic rules for the management of compensations between criteria. LCA's threshold values were defined by taking the extreme values obtained on a population of 5 vineyards, chosen to represent the diversity of viticultural practices for middle of Loire valley dry white wine grapes (France). Decision rules for compensations were decided to limit the compensation of a bad score on one criterium by a good one on another variable. CONTRA parameters that best correspond to these rules were then defined. The aggregation of results using CONTRA allowed an overall view of the LCA results of a system by producing a single synthetic score. Furthermore, CONTRA makes possible the analyze of the results more thoroughly backing up to the individual results and of environmental impacts and quality criteria, to identify elements to be improved and those to be maintained. Within this study, the use of CONTRA eased the comparison of environmental LCA results with another sustainability dimension, here the quality of the product.

TU258

Grape LCA : How to better assess organic and integrated technical management routes ?

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There is an increasing demand from society for informations on environmental impacts of products. In viticulture, there are a lot of different practices and there is a need for tools and methods that can give relevant informations on environmental impacts of these practices. Life Cycle Assessment (LCA) has been recently identified as a useful tool to develop more sustainable agricultural systems and as a relevant method to assess environemental impacts of agriculture on a plot scale. A method has been proposed by Renaud-Gentié et al. to assess environmental impacts of technical management routes (TMR) on a plot scale based on LCA. This study apply this methodology on two case studies and goes through methodological improvements needed for a better assessment of organic and integrated viticulture practices. Results show that the studied organic TMR has significantly more impacts on global warming potential, photochemical ozone formation potential and acidification potential. Concerning freshwater ecotoxicity potential and resource depletion, impact score difference between organic and integrated TMR is very small. The case study shows how current application of LCA methodology to viticulture is not sufficient to assess different types of viticulture. In this methodology, several major issues are not assessed fairly or not assessed at all. For example, adding a soil quality indicator among impact categories seems important as maintaining initial soil properties is a key issue for agriculture sustainability. Carbon sequestration in the soil is another important issue which is not taken into account in Grape LCA whereas existing good techniques in this field may be promoted by LCA. Concerning ecotoxicity, modelling of emissions to soil, water and air from copper and sulfur-based products (mainly used in organic agrilculture for pest management) doesn't exist. Finally, data used for fuel consumption of machinery are not totally satisfactory and can also be improved.

TU259

A new biophysical allocation in LCA of beef cattle coproducts: modelling energy requirements of body-tissues growth

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/ UMR SAS; L. Puillet, INRA, AgroParisTech, Université Paris-Saclay / UMR MSAR; J. Aubin, INRA , Agrocampus Ouest / UMR SAS

In agricultural life cycle assessment (LCA), the choice of allocation methods to spread out impacts between coproducts is an important issue, as they may induce different conclusions in impact levels. We proposed a biophysical allocation method to dispatch the upstream environmental burdens and the use of raw materials to the body-related coproducts of beef cattle production system at slaughterhouse stage. The method is designed to build a relationship between coproducts of the beef cattle production system and their associated net energy requirements for body growth. So it doesn't take into account the fate of the different coproducts, but only their building costs (i.e., energy needed for building tissues). A combination of metabolic growth model (Gompertz function) and energy model was used to estimate the energy requirements for body growth from birth to slaughter age. The allocation factors were calculated based on the energy requirements attributed to build body tissues characterized by their chemical compositions (protein and lipid) and. Finally, this method was compared with other allocation methods (e.g., physical, economic). The biophysical allocation reflects a physical and biological relationship between the coproducts as required by ISO standard. It provided a moderate allocation factor for human food due to their chemical characteristics compared to the other physical allocation methods. In addition, the data required is specific to species and less influenced within a predefined system than economic allocation. This study provides a generic and robust biophysical allocation method to handle the coproducts in beef cattle system. The method can be considered as an original contribution to the international debates on the allocation methods in LCA applied to livestock products, especially among the stakeholders of the meat value chains.

TU260

How to take into account the crop rotation into Life Cycle Assessment of crop at national scale ? Methodological development and results from ECOALIM project

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Animals' feeding contribute very significantly to the overall environmental impact of animal products. The French project ECOALIM aims to improve the environmental impacts of husbandries by optimizing their feed. This project (1) defines the environmental impacts of the production of raw materials for animal feeding basing on Life Cycle Assessment (LCA) and (2) optimizes the formulation of compound feed with environmental constraints in order to improve environmental footprint of animal products. Animal feeding optimizing could induce substitution from one crop product to another into the formulation. In the meantime, it is difficult to allocate the impacts of a production system to each crop in a crop rotation because emissions depend on practices implemented on the whole crop rotation and not only a single crop. The method to allocate emissions between crops and its results on 12 case studies are discussed here. Specific methodological developments were made in particular to allocate nitrate (NO_3^-) and phosphorus (P) losses between crops. NO_3^- leaching due to the nitrogen (N) fertilization of one crop depends on the applied quantity but also on the intercrop management and on the following crop. As P is immobile in the soil, some farmers use residual nutrients by applying P fertilizers to one crop only in quantities sufficient to supply the off take and needs of following crops. Hence, after assessment of different allocation rules, NO_3^- was allocated equally between crops within a same rotation and a specific rule was defined to allocate P in function of off take and needs. Organic N was allocated according to the rule used for Agribalyse. For each case study, LCA was applied on a basic scenario and on 3 crop management scenarios, aiming to reduce environmental impacts related to loss of nutrients and use of mineral fertilizer: 1) introduction of intermediate crops, 2) introduction of legumes in the rotation, or (3) organic fertilization (with 2 options for the choosen organic fertilizers in each case study, 3-1 and 3-2). LCA results show that if these rules seem pertinent to allocate benefits of some crop management between crops, they raise questions for some others (cf table 1). Finally, these results represent a step forward in order to take into account system production improvements in LCA of single crop but also show that some developments are still required as results obtained with a same method are very variable in function of hypothesis.

TU261

Biomarker analysis in soils of the Amazon rainforest after vegetation fire

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Biomarkers, also known as molecular markers, refer to compounds that have a relationship with their precursors and indicate the occurrence of a particular process within an organism. Recently, the evaluation of biomarkers in plants or soil has become a more common procedure, since the abundance of these compounds are sensitive to changes in the soil organic matter (SOM) caused by the use and management of soil. The content of this fraction comprises much of the soil aliphatic structures and may give information about the source of the organic matter present in soil (microbial origin or higher plants) and is therefore called biomarkers. Studies on soils biomarkers can contribute to the understanding of the dynamics and stabilization of organic matter, which is of great importance from an environmental point of view.The objective of this study was to determine the origin of organic matter incorporated in Amazon forest soils subjected to vegetation fire by analyzing the aliphatic biomarkers (*n*-alkanes) present in lipid extracts of soil samples. The study area is located at Embrapa-Acre in Rio Branco, Acre/Brazil and consists of primary forest which was partially burned in 2011. Samplings were conducted in September 2012 (one year after burning) and September 2014 (three years after burning) in primary forest areas (PF) and burnt forest (BF) at two depths: 0-5 and 100-150 cm. Approximately 4 g of sample was used for lipids extraction. The samples were extracted via soxhlet with dichloromethane/methanol (3:1 v/v) for 24 hours. Completed the extraction step, the lipid extract was concentrated in rotary evaporator. The extracts containing lipids were then subjected to fractionation using preparative liquid chromatography atmospheric pressure. The distribution of *n*-alkanes was determined by GC/MS. The SOM of the primary forest comprises contributions of microbial moieties and of higher plants along the whole profile. This similar distribution pattern of *n*-alkanes indicates that a percolation of SOM fragments produced at the surface to the deeper layers occurred. After vegetation burning, a lipids fraction original from higher plants are fragmented in smaller chains, enriching thus the proportion of C₁₆ to C₂₄*n*-alkanes. This effect was also observed at the deeper layer, suggesting that the percolation of SOM along the profile is a constant and intense profile.

TU262

Life cycle assessment of hemp concrete blocs

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Buildings notably contribute to global environmental negative impacts due to consumption of both embodied energy and natural resources as well as various emissions during their whole life cycle. It is therefore necessary to develop practices to reduce these impacts, mainly by reducing the part of non-renewable resource in material as well as by ensuring the lowest energy consumption possible during their lifetime. New developments in natural fibres and their use in insulating materials can lead to significant improvement in building environmental impact. For this purpose, assessment of environmental performance is needed to support both the design and the production of (new) fibre based insulation solutions. In this context, the Life Cycle in Practice (LCiP) project helps SMEs to reduce the environmental impacts of their products and services across the entire life cycle. Within the frame of this project, Isohemp (BE) hemp concrete bloc impact is evaluated in a cradle-to-gate LCA. Functional unit is a pallet of hemp concrete blocs ready for shipping. It represents about 1.3 m^3 of blocs. Hemp blocs are made by pressing a mix of hemp shives, hydraulic and hydrated limes, and water. Long term carbon storage due to lime carbonation induces a large benefit in Climate Change (CC) category. The balance of the CC indicator for hemp cultivation is also favorable due to carbon dioxide uptake by the photosynthesis occurring during the plant growth. Life Cycle Assessment of hemp concrete blocs ready to ship has also highlights some improvements that can easily be made at the packaging level in order to lower the global environmental impact and increase the sustainability of this insulation material. Data are processed in SimaPro 8 software, with Ecoinvent 3 and ELCD 3 databases, and analyzed with the CML IA method. This method is compliant with the indicators required by EN15804 standard in order to communicate on the environmental performance of Isohemp blocs.

TU263

A regionalised life-cycle analysis structure for sustainability assessment of bioenergy systems

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sustainability assessment of bioenergy systems is often challenged by various uncertainties including spatial variables. Life-cycle assessment (LCA) is a reliable methodology for sustainability assessment and it has been applied extensively to bioenergy and agricultural systems. The step-by-step structure of LCA is capable of taking into account environmental repercussions of the whole life cycle of bioenergy production, but the results are often only valid for specific case studies or too general to be used for regional decision making. By enhancing the generic structure of LCA and expanding the current datasets, we are proposing a more efficient procedure which is capable of producing region-specific results. The suggested framework includes techniques for making the life cycle inventories more relevant by regionalised compilation procedures. In addition, the framework integrates improved selection of appropriate impact assessment models. This methodology can specifically be used by local stakeholders or decision makers, who are considering producing energy biomass, as a guide for improving the environmental performance and efficiency of their activities.

TU264
Influence of crop management on water use of agricultural products. Case study: soybeans (Glycine max) in conventional tillage versus no-tillage in Argentina
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The aim of this study is to analyze the influence of tillage systems in assessing consumptive use of water in agricultural products. A new procedure which allows the incorporation of the water balance of the soil on the green and blue water footprints calculation is presented. For this, an adjustment of crop evapotranspiration and surface runoff is performed, taking into account the effects related to the presence of stubble, crop cover and surface roughness. The case study of soybean (Glycine max) in conventional tillage and no-tillage in Argentina constitutes the first application of this procedure. The results confirm that no-tillage reduces soil evaporation, prolongs the water storage period in the soil, delays the onset of the surface runoff and decreases runoff depth. This improves the water availability to satisfy crop needs and therefore increases the consumption of green water. The increased water availability increases the crop productivity, resulting in a diminution of green water footprint. If the soybean is grown under irrigation, the increase on the green water consumption corresponds to blue water savings. The considerations of tillage systems in assessing green and blue water footprints would be highly relevant in products derived from crops whose productivity is evenly influenced by hydric stress throughout the growing cycle. Likewise, tillage effects on the green and blue water footprints would be of greater magnitude for crops grown in regions with regular rainfall, or where the periods of greatest rainfall take place during the stage of crop development.

TU265
Modelling agricultural production: systematic analysis of secondary Life Cycle Inventories
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The analysis of agricultural production with life cycle based methodologies is very data demanding. In order to build comprehensive life cycle inventories, the use of secondary datasets is a common practice when primary data are not available. However, different inventory data and modelling approaches are used to populate secondary datasets, leading to different results. This can limit the perception of the reliability of the LCA tool especially when it is used for comparison purposes, e.g. among different food products and competing agricultural practices. We analysed the features of secondary datasets for agricultural production with the aim to identify how different inventory data and modelling choices can influence the LCA results. The outcomes of the analysis can support LCA practitioners in the selection of secondary datasets and proper interpretation of the results and can contribute to the challenge of defining a common modelling framework for agricultural production. Wheat grains production in France was assessed using three datasets from different databases (Agrifootprint v1.0, Ecoinvent v3.1 and Agribalyse v1.2). Firstly, we compared the system boundaries and the general assumptions. Secondly, we focused on the foreground systems by comparing inventory data, data sources and modelling approaches. Thirdly, we performed a contribution analysis on the impact assessment results to identify those modelling choices that contribute the most to differences in the results. We identified and assessed nine relevant elements: definition of system boundaries and modelling of agricultural practices; characteristics of inventory data; agricultural operations modelling; fertilisers' application and fate; plant protection products application and fate; heavy metals inputs to the agricultural system and fate; irrigation assumptions; land use and transformation. These elements included crop-specific data, such as the amount of inputs provided to the field, and database-specific modelling approaches, common to all the datasets modelling agricultural production within the database. The three datasets differed greatly with respect to those elements. Hence, we drew recommendations from the analysis of database-specific modelling approaches, supporting LCA practitioners in the selection of the datasets coherently with the goal and scope of their study and in the interpretation of the results and fostering the development of common rules to model agricultural production.

Ecotoxicological assessment and water quality monitoring in support of marine and freshwater legislation in Europe (P)

TU266
PAH exposure, genotoxicity and histopathological alterations in Scottish flatfish
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The UK has long been committed to monitoring and assessing the quality of the marine environment, and has a vision for its seas to be Clean, Safe, Healthy, Biologically Diverse, and Productive. Along with other countries, the UK reports marine monitoring data to international bodies such as the International Council for the Exploration of the Sea (ICES) and the OSPAR Convention for the Protection of the NE Atlantic which will be used in assessments under the EU Marine Strategy Framework Directive. As part of the Scottish monitoring programme flatfish blood samples were collected from the Firth of Forth, Moray Firth, offshore Scottish east coast and the North Minch. Samples were assessed for the presence of micronuclei, nuclear buds and binucleated erythrocytes and the results compared to observed liver histopathologies and exposure to polycyclic aromatic hydrocarbons, as indicated by biliary 1-hydroxypyrene concentrations; the significance of the data was assessed using OSPAR Assessment Criteria. The differences between polluted sites and 'cleaner' offshore site were examined, alongside differences between European plaice (*Pleuronectes platessa*) and common dab (*Limanda limanda)*. The highest biliary PAH concentrations and greatest frequency of micronucleated erythrocytes were observed in flounder from the industrialised lower Forth estuary; early toxicopathic lesions and foci of cellular alterations were also more commonly observed in fish from the Forth. PAH exposure and toxicological responses were lower in offshore dab, particularly those from the North Minch (NW Scotland).

TU267
ANALYSIS OF COMMUNITY-LEVEL MESOCOSM DATA BASED ON ECOLOGICALLY MEANINGFUL DISSIMILARITY MEASURES AND DATA TRANSFORMATIONS
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The Principal Response Curves (PRC) method is a constrained ordination method developed specifically for the analysis of community data collected in mesocosm experiments which provides user-friendly summaries and graphical representations of the data. The PRC method is based on redundancy analysis (RDA) usually performed on log-transformed abundance data. The log-transformation is used to lower the weight of the most abundant species in the analysis and implies that the variations in abundance are scaled to the total abundance of each species. Many different measures to calculate dissimilarity between samples on the basis of the abundance of each species have been developed, in particular in the field of ecology. The measure of dissimilarity between samples and the data transformations have a very large impact on the results of the multivariate analysis. The Euclidean distance between samples is implicitly used in PRC, but in the field of ecology, many more dissimilarity measures are currently used. Distance-based redundancy analysis provides a basis for integrating more ecological meaningful distance measures into the PRC than Euclidean distance alone. In this paper, we investigated the ordinations produced with a small selection of ecological meaningful dissimilarity measures, namely the Euclidean distance on log-transformed data, the Hellinger distance, and the Bray-Curtis dissimilarity on raw and log-transformed data. We compared unconstrainted ordination as well as PRC results obtained with these dissimilarity measures for two different macro-invertebrate community datasets, resulting from experiments performed with anti-inflammatory drug diclofenac and the insecticide chlorpyrifos. In both cases, the ordinations obtained with unconstrained ordinations and RDA were similar, indicating that, once time effects had been partialled out, the treatment effects were the major source of variability in the data. In the diclofenac dataset, the Hellinger distance produced interesting significant results that completed those obtained with the Euclidean distance on log-transformed data. The chlorpyrifos dataset showed similar results for the different dissimilarity measures, with slight differences in the number of significant dates. The fraction of the total variance belonging to between-replicate and time-dependent variation appeared to be an important criterion in the selection of a dissimilarity measure.

TU268
A complete pesticide monitoring in small Swiss rivers over a growing season applying a screening method and a continuous sampling strategy
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Agricultural pesticides are regularly found in surface waters at concentration levels that raise ecotoxicological concerns. Due to large fluctuations in concentration over time and the potentially high number of pesticides in agricultural watersheds, it is difficult to obtain a comprehensive overview of the actual pollution level. This collaborative project between research and Swiss federal and cantonal authorities aimed for a comprehensive analysis of pesticide pollution in five small agricultural streams to address this knowledge gap. The five rivers are located in catchments with intensive agriculture covering a wide range of crops, such as grains, vegetables, vineyards and orchards. Twelve-hour composite samples were collected continuously from March until the end of

August with automatic sampling devices, resulting in 360 samples per site. Using precipitation and water level data, we differentiated between discharge events and low-flow periods. Samples taken in dry weather between rain events were pooled for the analysis. This procedure resulted in a complete concentration profile over the entire monitoring period covered by 60 samples per site. The analysis, using liquid chromatography coupled to high resolution mass spectrometry (Orbitrap technology), involved a target screening of 248 pesticides including fungicides, herbicides, insecticides, as well as important transformation products. Data on the total number and distribution of pesticides, their detection frequency, crop specific applications and concentration time profiles will be presented. Preliminary results indicate substantial pesticide exposure since at least 20 different compounds were detected in each sample. One sample even contained a mixture of 80 pesticides. The majority of concentrations were in the low ng/L range but concentrations of a few compounds were very high (several micrograms/L) during discharge events as well as during low flow conditions and exceeded environmental quality standards (EQS).

TU269

Analytical developments to study diclofenac and some of its transformation products in a contaminated model ecosystem: analysis in bivalve, fish, sediment and watercress

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Pharmaceuticals are widely found in the aquatic environment, essentially at trace levels, nevertheless long-term exposure can have negative effects on biotic communities due to their intrinsic biological activity. Diclofenac (DCF) is one of the most frequently detected human pharmaceuticals in water and it has lately been included on the “watch” list of the European Union. However little data is available on the detection of this substance and its transformation products in aquatic organisms. In this context, analytical methodologies based either on QuEChERS or on ASE extraction followed by LC-MS/MS analysis were developed to quantify traces of DCF along with nine of its biotic and abiotic transformation products in bivalve, fish, sediment and watercress collected from lotic mesocosm experiments. The analytical protocol was first independently optimized and validated for each matrix of interest: sticklebacks, zebra mussels, sediment and watercress. For the optimization steps, uncontaminated matrices, corresponding to the matrices introduced into the mesocosms before the contamination with DCF were used. Considering the great diversity of transformation products and metabolites, a focus was made in this study on chemically stable and commercially available substances. Next target compounds were measured in samples collected from 6-months mesocosm experiments where DCF was continuously introduced in triplicate channels at three concentrations (5; 0.5 and 0.05 μg/L) and three more channels without DCF were used as control ones. DCF was quantified in zebra mussels and sediment for both highest exposure conditions and in watercress solely for the highest concentration. In sticklebacks DCF resulting concentration was inferior to our quantification limits probably due to rapid depuration as fishes were collected one week after the end of experiments. Among the selected metabolites, 4’OH-DCF was the most often quantified : it was observed in sticklebacks, sediment and watercress for the highest exposure concentration. The metabolites DCF-lactam and 5-OH-DCF were also measured as well as the 2-indolone either in sediment, watercress or bivalves. In this presentation, the optimization of the extraction and clean-up steps will be described. The results of the quantification of DCF, along with nine of its degradation products and metabolites, in organisms and watercress collected from mesocosms will be presented.

Science Integrity and Publication Bias (P)

TU270

What if Science follows Policies? The case of advanced biofuels

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This article presents the results of a literature review performs with a meta-regression analysis (*MRA*) that focuses on the estimates of advanced biofuel *GHG* emissions assessed with a Life Cycle Assessment (*LCA*) approach. 47 *LCA* studies are included in the database, providing *593* estimates. Each study estimate of the database is characterized by *i)* technical data/characteristics, *ii)* author's methodological choices and *iii)* typology of the study under consideration. 82% of these *GHG* emissions results from North American (*NA*) authors are compliant with their more restrictive *GHG* emissions minimum threshold whereas it is the case for only 59% from European (*EU*) authors. This systematic difference between *NA* and *EU* observations may come from the use of a different set of technical variables, for instance, but it may also reveal the existence of a potential publication bias in the literature. Then, we conducted a *MRA* in order to identify the key factors influencing the results. Among others, we set the geographical location of authors as well as some technical data (type of technology, mass yield, etc) as explanatory variables. Results show that the geographical location of authors always has an influence on the *LCA GHG* emissions what ever the sample considered and that *NA* observations are always lower than *EU*. Investigating more in-depth a potential bias linked with the geographical location of authors, the Funnel graphs reveal the existence of an asymmetrical publication bias (*PB*) of *NA vs. EU* studies. This *PB* is then statistically tested by proposing an adaptation of the Funnel Asymmetry Test (*FAT*) of Stanley (2005). This test reveals a systematic *PB* of *NA* studies to publish results under the minimum thresholds for life cycle *GHG* emission savings specified by the *RFS2*, which is not the case for *EU* Studies. This result is of primary importance as it highlights the influence of the design of some public policies on the very scientific research and/or publication process. Policy makers have to be aware of this potential bias when designing new policies. Our results indicates a hierarchy between advanced biofuels as mean values of their LCA GHG emission weighted by the influence of its main drivers range from 19.5 (BtL) to 60.0 (G3) gCO_2eq/MJ of biofuel. This range of values is lower than fossil reference (about 83.8 in gCO_2eq/MJ), but only G2 Ethanol and BtL do comply with the GHG emission reduction thresholds defined in the US and EU regulations.

TU271

(No-)Effects of Polyoxometalates Encapsulated in Silica Nanoparticles in the soil invertebrate Enchytraeus crypticus

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Polyoxometalates (POMs) are metal-oxo clusters that have been investigated for several applications in material sciences, catalysis and biomedicine and have gained increasing interest in the field of nanotechnology as nanocarriers for drug delivery. Associated to the increasing applications there is the need for information regarding the effects on the environment of these compounds, which is completely absent in the literature. In the present study the effects of europium polyoxometalates encapsulated into silica nanoparticles (Eu-POM/SiO_2 NPs) were assessed on the soil representative *Enchytraeus crypticus*. The individual materials were also assessed (Eu-POMs and SiO_2 NPs). Toxicity was evaluated in various test media with increasing complexity: water, soil:water extracts and soil. Toxicity was only observed for Eu-POM/SiO_2 NPs and in the presence of soil components. Despite the fact that effects were observed for concentrations higher than current PECs (Predicted Environmental Concentration), attention should be given to the growing use of these compounds. The present study shows the importance of assessing effects in soil media, also compared to water. Moreover, results of ‘no effect’ are critically needed and often unpublished. The present contributes to the improvement of the OECD guidelines for safety of manufactured nanomaterials on environmental toxicity in the soil compartment.

TU272

The axe over the academic head: science integrity for ecotoxicology in a high-pressure, science communication era.

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In science, integrity is critical, not only for the individual scientist but for public and political confidence to drive progression in knowledge. Ecotoxicology has developed into a fashionable field which is governed by public and political awareness, scientific bias for ‘pet’ pollutants and to a certain degree, new technologies which can lead to over-emotive scaremongering. Coupled with a high pressure working environment to deliver both funding and high quality publications, this creates a perfect storm to erode away science integrity, enhance conscious and unconscious bias and cutting corners in experimentation. This has recently been highlighted in the requirement to test the 'repeatability' of experiments across a number of scientific, including medical fields. To succeed as a young and established scientist in today's world, one must be able to publish regularly in high grade publications and show consistent funding successes beyond normal scholarly activities. There is an argument that to get your science published it must deliver significant results with impact resulting in publication bias. The consequences for not achieving this for many is job loss or at best the 'perceived' downward trajectory of teaching and administration. This paper highlights personnel observations of key pressure points on scientists whereby these pressures to consciously or otherwise cut corners is a growing concern for our field. In addition, whilst the #scicomm era is an excellent advancement in delivering factual science and impact right to the door of appropriate stakeholders, it also has the potential to allow people to drive their own agendas and ultimately harm public perception in science. There is a requirement to re-establish scientific integrity back onto the syllabus in science education; encourage publication of negative results and reduce pressure on academics to deliver ‘fast science’.

Tendency towards higher complexity in environmental risk assessment of Plant Protection Products: to accept or to

avoid? (P)

TU273
Sequences of pesticide applications: Is the prospective RA representative for the agronomic reality?
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The prospective ecotoxicological risk assessment (RA) conducted during the registration of plant protection products (PPP) is a complex procedure, based on tiered assessments of toxicity and exposure. The exposure profile is directly affected by the application pattern of the PPP by the farmer. Thus, in order to assess the ecotoxicological risks of PPP, information on the PPP application is needed. Application parameters, i.e. application rate, number of applications and interval between applications, have to be established by the regulatory authority for each registered indication, i.e. use of a PPP on a specific crop and pest. Application parameters are established based on the following criteria: ensuring effective treatment of pest and reducing resistance risk, while keeping the risk of humans and the environment in an acceptable range. In this study we investigated, whether the prospective environmental RA is in accordance with the agricultural practice. Since 2009, data for PPP use of approximately 300 farmers are recorded within the framework of agri-environmental monitoring (AEM) in Switzerland. Specific information per lot of land is being collected about the actual use of PPP, including application rates, treated crops and application times. Based on this data set we analysed the application of PPPs, considering application rates, number of application and intervals between applications. These data from the AEM were compared to the authorised uses of PPP. First results indicate a high variability in the analysed AEM application parameters. However, despite their heterogeneity, the AEM data seem to largely be in accordance with authorised uses, considering parameters set in the registration such as application rates, number of applications and intervals. A high complexity of a RA is only justified if it does reflect the reality better than a less complex RA. However, by stating the parameters along with the registration a higher transparency and reliability may be achieved. General questions as to whether further standardisation and stating of application parameters is necessary and feasible or how sequential PPP applications could be considered in the registration are discussed.

Chemical Transport via the Global Food System (P)

WE001
Development of a method to extract chemicals from food for toxicity testing
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Many environmental chemicals are known to be present in or on food. In particular, low income countries can suffer from the application of mostly banned and toxic pesticides in agriculture, combined with misapplied usage, which can lead to high food contamination. Owing to potential human health concerns, residues in food are generally identified with targeted chemical analysis. There is increasing awareness of assessing the biological effects of contaminants present in food, but few studies have applied *in vitro* bioassays for monitoring food based chemicals. To overcome this problem, the present study focuses on the development of a simple extraction method for bioanalytical testing of chemicals in food. The bioassays were selected throughout the cellular toxicity pathway, including induction of xenobiotic metabolism and cytotoxicity. The Chemically Activated Fluorescence gene expression assay (AhR-CAFLUX) detects compounds activating the xenobiotic mechanism via activation of the arylhydrocarbon-receptor (AhR). While this assay is typically used to screen for dioxin-like compounds, a set of other compounds, such as polychlorinated biphenyls (PCBs) and some pesticides, are known to activate the AhR. To screen for non-specific acting toxicants, the bacterial luminescence toxicity screen (BLT) assay was applied. This assay reflects the response of all chemicals present in a sample, causing different damages on bacterial cell viability. Liquid-liquid extraction, which was selected due to reduced solvent demand and rapid sample treatment, was applied for a range of food types. Additionally, washing water was also screened for chemicals to assess food processing. Regarding extraction efficiency, the method seems to be more efficient for high watery samples (strawberries, tomatoes) instead of fatty (fish, meat) and high fiber (apples) samples. Hence, higher biological effects were obtained for watery samples in the studied assays, including response of the washing water. Future work should focus on method improvement of the liquid-liquid extraction to address the urgent need of a reliable hazard assessment of food for low income countries including toxicity testing.

WE002
AMINOGLYCOSIDES ANTIBIOTICS RESIDUES ANALYSIS IN BOVINE MILK AND BOVINE, SWINE AND POULTRY MUSCLE BY LC-MS/MS AND LC-QTOF-MS: A SIMPLE AND FAST NON SPE METHOD
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The presence of several different substances in animal products has been a major public concern. The use of antibiotics drugs as veterinary medicines might lead to the development of bacterial resistance, which might undermine the efficacy of these drugs in human use. The Brazilian government implemented a control plan that assesses the residues of veterinary medicines in animal products. The aminoglycosides are antibiotics that have been extensively employed in animal husbandry for the treatment of bacterial infections, but also as growth promotion. The European Union has issued strict maximum residue levels (MRLs) for aminoglycosides in several animal origin products. An analytical method has been developed for determination of ten aminoglycosides (spectinomycin, tobramycin, gentamicin, kanamycin, hygromycin, apramycin, streptomycin, dihydrostreptomycin, amikacin and neomycin) in bovine milk and bovine, swine and poultry muscle. A simple, fast, cheap and ecofriendly extraction method with non SPE steps was developed using trichloroacetic acid and clean up with low temperature precipitation and C18 bulk. A 2^4 factorial design with center point was performed to assess changes in the steps of the extraction process and the best condition was adopted. The LC–MS/MS and LC-qTOF-MS methods were validated according to the European Union Commission Directive 2002/657/EC. Good performance characteristics were obtained for recovery, precision, calibration curve, specificity, decision limits (CCα) and detection capabilities (CCβ) in all matrices tested. The data detection limit (LOD) and limits of quantification (LOQ) was established from MRL ranging from 5 to 100 ng g^{-1} for LOD and 12.5 to 250 ng g^{-1} to LOQ. Good linearity (r^2) above 0.99, considering three different days, for all aminoglycosides was achieved in concentrations ranging from 0.0 to 2.0 x MRL, noting that all curves have been made into their own matrices in order to minimize the effects matrix which are intrinsic to the cases studied in this work. Recoveries ranged from 36 to 98% and the coefficient of variation from 0.9 to 20.2%. The CCβ values obtained in qualitative method were between 25 and 250 ng g^{-1}, considered satisfactory for the analytes in those matrices. The proposed method proved to be simple, easy, and adequate for high-throughput analysis of a large number of samples per day at low cost.

WE003
Levels of pharmaceuticals and endocrine disruptors in commercially available seafood before and after cooking
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Research has shown that eating fish and selfish regularly is beneficial to our bodies in many ways. However, it can also be a source of harmful environmental contaminants with potential to impact negatively on human health. The presence of "contaminants of emerging concern" like pharmaceuticals and endocrine disrupting compounds in seafood for human consumption needs to be monitored. Besides, to study the effect of cooking and processing on contaminants levels in seafood becomes a relevant issue since it has been observed that an increase in the concentration may occur. Therefore, the main objectives of this study were to detect the possible presence of pharmaceuticals and endocrine disruptors in seafood, to investigate the effect of cooking on contaminants levels, and to establish whether the measured concentrations were below the maximum levels set by the European Union. Besides, a risk assessment was performed in order to evaluate the human health impact of the contaminants present in seafood. An European sampling survey of seafood collected in different European regions have been carried out.Several commercial seafood species including seabream, mackerel, tuna, salmon and mussels have been sampled on different seasons (autumn and spring time) and geographic locations (Spain, Italy, Portugal, Denmark, Norway, Netherlands and France). Based on previous results a list of priority PhACs and EDCs was targeted. The presence of compounds such as diclofenac, diazepam, sotalol, carbamazepine, citalopram, venlafaxine, azithromycin and sulfamethoxazole, TBEP, bisphenol A, and triclosan was researched. In the majority of the samples analysed PhACs compounds were nor detected or below method quantification limits. In few ocassions positive

identification of some pharmaceuticals was done reaching up to 11.7 ng/g dry weight of sulfamethoxazole in mussels from Netherlands. Regarding EDCs methylparaben, triclosan, and bisphenol A were frequently detected in seafood. Besides, an increase in these contaminants levels was observed after cooking by steaming. The highest level measured correponded to bisphenol A in canned tuna reaching up to 69.1 ng/g dw. Due to their presence and levels in seafood methylparaben, triclosan, and bisphenol A were selected for performing risk assessment, and for researching whether intake of these contaminants through seafood consumption involves a risk for human health. This piece of research is currently ongoing and results will be presented at the meeting.

WE004

Heavy metals in freeze dried milk from Brazil

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The production of milk in Brazil is one of the most important rural activity involving and more than 30 billion of liters being produced each year. Essential food for different but important population groups like children and the elderly, milk is an invaluable source of important micronutrients like zinc, copper and iron. However mineral supplementation or even corrosion in metallic equipment may also represent a source of non-essential and toxic elements, like mercury, cadmium and lead that may sometimes be a route of contaminants to human beings. Since soil particle ingestion is frequently observed during cattle grazing, our objective with this study is to determine if the levels of this elements in freeze dried milk can reveal a route for the transference of the selected metals to man. Around two grams of freeze dried milk where analyzed in triplicates after calcinations (450 ºC) and acid digestion in hot-plates. All measurements were performed at the Radioisotopes Laboratory of the Biophysics Institute of the Federal University of Rio de Janeiro. In spite some cases of contamination by Fe in some imported brands of milk in powder; the recurrent problem in Brazil is milk adulteration by addition of caustic soda and formaldehyde in the production of UHT milk. As expected, there is a higher contribution of Zn in the overall results, indicating once more that milk is an important source of this micro-element. Although most farms are equipped with stainless steel devices for refrigeration and storage, the use of less noble steel more fond of corrosion can be considered a source of toxic metal contamination, especially Ni and Cr. As for Pb, this metal may be present in anticorrosive paint. In the present study, with only few exceptions for Cr and Pb, the determined values were not higher than the provided maximum allowance for the agricultural Brazilian legislation. In the absence of new voltametric and anodic stripping methods for direct measurements of the metallic contents of milk, the so called *old fashion* methods are still a choice and can help in the characterization of the inorganic contamination of dairy products. More research is needed to clarify the relation on proper maintenance of metal equipments, soil contamination and milk quality in order to acquire better standard production parameters for milk source qualification, in the view of an organic farmer green product verification/certification.

WE005

UV filters maternal transfer through breastfeeding

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Organic UV filters are the preferred protection against adverse effects of UV radiation in all kind of cosmetics, as well as in common materials and industrial products. This fact leads to a continued human exposure. Many studies show the tendency of UV-F to bioaccumulate in living organisms due to their lipophilicity and stability versus biotic degradation [1]. The extended use and the ubiquity of these compounds in the environment make necessary studies on the bioaccumulation in human tissues and elimination from the body of these compounds through biological fluids. Breast milk is a biological fluid that has a fundamental role in new born nutrition and has direct impact on growth, neurodevelopment and health [2]. Breast milk is a compositionally variable fluid that is known to change during the lactation period, and it is highly influenced by maternal dietary intake and life style. In this framework, the aim of this study was to assess the potential maternal transfer of UV filters to newborn through breastfeeding. BP3, BP1, 4HB, 4DHB, OD-PABA, OC, EHMC, Et-PABA , BZT, TBHPBT, UV320, UV329, 4MBC and MeBZT were the UV filters investigated. Breast milk samples, provided by mothers from Barcelona were first extracted by USE and after internal standard addition, analysed by Turbulent Flow-Chromatography-HPLC-MS/MS. Results indicated that 7 out of the 14 UV filters investigated were detected in the 79 milk samples analysed. 28 samples were positive in at least one compound. BP3 was the most frequently detected compound (68%) and the one observed at the highest concentration, 1599.8 ng/g milk. Two human metabolites of BP3, 4HB and 4DHB, were also identified in the samples. Finally, we concluded that breast milk samples contained a number of UV filters, mostly BP3 and two of its human metabolites, which suggest that not only UV filters are accumulated but also the more polar metabolites formed. During breastfeeding mother “eliminates” its own contamination but transfer it to the neonate. References [1] Kim JW, Ramaswamy BR, Chang KH, Isobe T, Tanabe S. 2011. J Chromatogr A 1218: 3511-3520. [2] Miller, E. M.; Aiello, M. O.; Fujita, M.; Hinde, K.; Milligan, L.; Quinn, E. A. Am. J. Hum. Biol. 2013, 25, 1−11. *Acknowledgement* - The authors thank the Generalitat de Catalunya (Consolidated Research Group “2014 SGR 418 - Water and Soil Quality Unit”) and MINECO (Project: Transferencia de filtros solares UV madre-hijo a través de la lactancia materna (SOLA)).

WE006

Polybrominated Diphenyl Ethers and Polychlorinated Biphenyls in Breast Milk from Serbia First-Time Mothers

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Polybrominated diphenyl ethers (PBDEs) and polychlorinated biphenyls (PCBs) are two highly lipophilic classes of persistent organic pollutants (POPs) able to resist degradation and with the ability to bioaccumulate through the food chain. The international community has responded to the threat from POPs by negotiating a global treaty, the Stockholm Convention on POPs, with the objective of protecting human health and the environment from POPs. Human milk may provide a significant source of exposure to POPs in early life, and data on levels of POPs in breast milk from different regions of the world have been generated in many publications. However, a little is known about levels of POPs chemicals in milk of Serbian mothers, especially concerning PBDEs. Objective of study was to investigate contamination of PBDEs and PCBs in breast milk from Serbia. A total of 11 breast milk samples from the first time mothers were collected in Department of Neonatology in Belgrade, Serbia from November 2014 to April 2015. Total lipids from breast milk samples were extracted by Soxlet method (ISO 8262-1) and subsequently the PCB and PBDE congeners were analyzed by HRGC/HRMS. Average concentration of PBDEs in samples was 753,8 pg/g fat, with highest share of BDE #47 more than 40%. Average concentration of PCBs in samples was 79,8 ng/g fat, with highest share of PCB #153 more than 14%. The PBDE (#17, #28, #47, #99, #100, #153 and #154) and PCB (#28, #52, #77, #81, #101, #105, #114, #118, #123, #126, #138, #152, #156, #157, #167, #168, #180, #189) contamination in breast milk as well as congener profiles was comparable to those in other neighbour countries in region. These results represent first published data of PBDE contamination of human milk in Serbia. Considering human milk’s nutritional and immunologic effects, society must take steps to decrease human exposure to these chemicals by restrictions in their usage, while continue encouraging women to practice breastfeeding.

WE007

Using RISK21 matrix to assess the potential human health risk of DDT exposure from flower tea

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The Health and Environmental Science Institutes-coordinated RISK21 is an integrated evaluation strategy which provides a straightforward, efficient, systematic, and transparent way for health risk assessment. RISK21 takes advantage of existing information to graphically represent the comparison of exposure and toxicity data on a highly visual matrix, termed RISK21 matrix. Recently, the long-banned pesticide DDT (dichlorodiphenyltrichloroethane) and its metabolites were found in the rose leaves as the flower tea drinks sold at Taiwan's popular tea chain store. The purpose of this study was to adopt RISK21 approach to assess the degree of health risk of DDT exposure from drinking flower tea or directly consuming rose leaves. Based on the foundation of risk assessment, we integrated the estimates of age-specific total daily intakes (TDI) of DDT with toxicity data on liver lesions and developmental toxicity to assess the level of health concern by RISK21 matrix. The TDIs through direct intake of flowers for various age groups were estimated based on the concentrations of DDT, DDD (dichlorodiphenyldichloroethane), and DDE (dichlorodiphenyldichloroethylene) in rose leaves as well as age-specific intake rate of flower tea, whereas additional factors of compound-specific water solubility and dilution factor on the flower tea were taken into account to calculate the TDIs from flower tea drinks. By considering the uncertainties of TDI estimates and toxicity estimates, Risk21 matrix shows that drinking DDT-contaminated flower tea seems unlikely to constitute a public health and safety concern for all age groups due to the extremely low exposure level. However, the human health risks of DDT exposure are alarming from a conservative point of view in regards to direct intake of flowers. RISK21 matrix is an easy-to-use tool that not only promotes the understanding of risk assessment but also fosters the risk communication.

Interactions between traditional, novel and green carbonaceous materials and contaminants (P)

WE008
Removal of 2,4-dinitrotoluene and 2,4-dichlorophenol with zero-valent iron-embedded biochar
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Removal of 2,4-dinitrotoluene (DNT) and 2,4-dichlorophenol (DCP) with zero-valent iron [Fe(0)]-embedded biochar was investigated as a possible in-situ remediation process. Fe(0)-embedded biochar was synthesized using rice straw via slow pyrolysis and its properties were characterized. DNT and DCP were chosen as model contaminants and their kinetics and mechanisms were examined through a series of batch experiments. Compared to reduction control by Fe(0) and sorption control by biochar, the removal by Fe(0)-embedded biochar was significantly enhanced, indicating that biochar plays a role of sorbent as well as electron-transfer mediator. Increasing pyrolysis temperature resulted in promoting the removal rate of the contaminants, suggesting that rather than surface functional groups, aromaticity of biochar may account for the catalytic role. According to yields of reduction products, the presence of biochar showed difference reduction pathways. Applicability of Fe(0)-embedded biochar in natural and engineered systems was also discussed.

WE009
Supporting of PAHs bioavailability and ecotoxicity reduction by nano zerovalent iron (nZVI) and biochar in industrially contaminated soils
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Polycyclic aromatic hydrocarbons (PAHs) are organic contaminants which are harmful for humans and other organinsms. One of the source of this contaminants is industry e.g. coal and bitumen production. The emitted PAHs can migrate into soil where they can accumulate in biota and negatively affect organisms. Due to this fact, there is a need of providing efficient tool for remediation of industrially contaminated soils by PAHs. One of the current remediation strategy of contaminated sites by PAHs is using a biochar as a sorbent for PAHs immobilization. To enhance the biochar PAHs reduction in contaminated soils, we supported soil remediation by nano zerovalent iron (nZVI), known from its effectiveness in the organic contaminants degradation. Therefore, two types of soils were investigated in this study: 1) KOK soil - sampled from the area of coking plant (Dabrowa Górnicza, Poland), and 2) POPI soil – sampled from the area of bitumen processing plant (Wólka Lancuchowska, Poland). Moreover, biochar produced from the wheat straw (BCS) and nano zerovalent iron, were used. Each soil were amended by BCS or/and nZVI in few variants: 1) by nZVI with a dose of 2 and 10 g_{nZVI}/kg_{soil} as a water suspension, 2) by biochar BCS with a dose of 5% w/w and equal amount of water needed for preparation of nZVI suspension, and 3) by nZVI with a dose of 2 and 10 g_{nZVI}/kg_{soil} as a water suspension together with biochar at a dose of 5% w/w. Pure soils samples amended with water as in 2) were taken as a control. Prepared samples were rolled end over end at 10 rpm for 7 or 30 days in dark and at room temperature. After 7 and 30 days, chemical and ecotoxicological analysis were carried out. Freely dissolved concentration of PAHs (C_{free}) was assessed by polyoxymethylene (POM) and ecotoxicity was determined using *Vibrio fisheri* bacteria (Microtox). Application of pure biochar and biochar together with nZVI to contaminated soils decreased a PAHs C_{free} and its potential environmental risk to the organisms. We also observed negative effect of decreasing bacteria bioluminescence after pure nZVI amendment to soils (especially in case of KOK soil). Nevertheless, combined addition of biochar and nZVI resulted in elimination of toxic effects of nZVI. Moreover, this variant in most cases showed the highest reduction of ecotoxicity and also provoked stimulation effects of bioluminescence in both soils.

WE010
Sorption of aromatic hydrocarbons to biochars
M. Kah, H. Sun, T. Hüffer, T. Hofmann, University of Vienna / Department of Environmental Geosciences
Many studies have demonstrated the strong sorption potential of biochars towards organic contaminants. Our ability to predict the extent of sorption for a given situation is however very limited, mainly because the effects that sorbate and sorbent properties have on sorption remain poorly understood. With the aim to support the development of predictive approaches, we systematically compared the sorption behavior of a series of biochars produced from different feedstock and at different pyrolysis temperature. All sorbents were extensively characterized and cover a wide range of properties that were previously identified as possible drivers for sorption (e.g., elemental composition, surface area, micro and mesoporosity). A series of aromatic hydrocarbons covering a range of molecular sizes were selected as sorbates (i.e., from benzene to pyrene). The sorption behavior of aromatic hydrocarbons is relatively well understood and allows investigating how the surface and porosity characteristics of the biochars affect sorption behavior. Sorption isotherms were measured across a concentration range covering several orders of magnitude. The Freundlich model fitted all isotherms very well, and non-linearity significantly increased with the pyrolysis temperature. The dual-mode model also proved useful to fit sorption isotherms and compare the contributions of solvent-driven and sorbent-driven processes (described by linear and Langmuir model, respectively). Multivariate analyses were also applied to identify the factors driving the great differences in sorption observed. Overall, the results provide a better understanding of the effects that biochar properties have on sorption behavior and thus possibly identify groups of biochars, whose sorption behavior could be described using common approaches.

WE011
Adsorption of Polychlorinated biphenyls to activated carbons with different surface and textural properties
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Activated carbon is a commonly used sorbent for the removal of organic pollutants from waters and industrial effluent streams. Although several studies have been conducted on the removal of persistent organic pollutants (POPs) from aqueous solutions using activated carbon, very little attention has been given to polychlorinated biphenyls (PCBs). In addition, the sorption efficiencies of PCB congeners on activated carbon with different surfaces and/or textural properties have not been fully explored. In this study, the sorption of PCB congeners from aqueous solutions to powdered activated carbon (PAC) or granular activated carbon (GAC) was studied experimentally using batch adsorption. The five target PCBs under investigation were PCB -1, 3, 7, 14 and 12. The efficiency of solid phase extraction (SPE) for pre-concentration was assessed using C18 (EC) or florisil SPE cartridges. Results obtained showed that C18 (EC) SPE cartridges provided higher extraction efficiencies (38 - 46 %) for the target PCBs compared to florisil SPE cartridges (9 -16 %). Investigation of the removal efficiency of PAC and GAC for PCB congeners was studied using a fixed mass of sorbent and varying initial PCB concentrations. GAC was found to have higher removal efficiency (33 – 63 %) for the target PCB congeners compared to PAC (50 – 96 %). Langmuir and Freundlich adsorption isotherms were applied for the analysis of equilibrium data. The correlation coefficients (r^2) obtained showed that the Langmuir model, in the linear form, adequately fitted experimental data for both PAC and GAC. The total PCB adsorption capacities of PAC and GAC obtained using predicted q_m values from the Langmuir model were 26.93 and 26.2 mg/g respectively.

WE012
Effects of Carica papaya seeds biochar and its Goethite modified amendments on Lead mobility in soil
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Presence of heavy metals (HM)-most especially Pb, in the environment has been worrisome because of its health implications on man and other biota. The labile fraction of HM in contaminated soils is however the most important. It is the portion that is readily available for plant uptake, thus accumulates in plants and eventually gets to humans who consume the plants. Therefore, any efforts that could lead to immobilization of HM in contaminated soils should be of uttermost scientific interest. This study reports the effects of Carica papaya seeds biochar (CB) and Goethite modified Carica papaya seeds biochar (GCB) on the mobility of Pb in a contaminated soil from the abandoned dump site of the defunct Exide-battery Manufacturing Company at Lalupon, Southwestern Nigeria. Ten treatments pots (in duplicates) were set up with the soil sample. First pot contained the sample at 0% amendment, and this served as the control. The second, third and fourth pots were amended with CB at 2%, 5% and 10% levels respectively; the fifth, sixth and seventh pots were amended with 5% Goethite modified CB (5%GCB) at 2%, 5% and 10% levels respectively, while the eighth, ninth and tenth pots were amended with 10% Goethite modified CB (10%GCB) also at 2%, 5% and 10% levels respectively. All treatments were brought to the field capacity with deionized water, and then incubated at room temperature for 30 days. The field capacity moisture was maintained throughout this period. At the end of the incubation period, 1g of the sample was extracted with 1M $MgCl_2$ solution, and the Pb content was measured with AAS. The $MgCl_2$-extractable Pb content of the control was found to be 1680mg/Kg. This was reduced by 80-89% by CB, 90-98% by 5%GCB, and 70-90% by 10%GCB. In conclusion, all the additives significantly reduced the bioavailable Pb in the soil, with the 5%GCB being the best, followed by CB. This has opened further opportunities for similar researches with other agricultural biomass. Keywords: Heavy metals, Carica papaya, Biochar, Goethite

WE013
Sorption of substituted aromatic hydrocarbons by fullerenes - Influence of functionalization on molecular interactions
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The interpretation and prediction of molecular interactions between organic compounds and carbon-based nanomaterials is of major importance to understand phase transfer processes in environmental matrices and to assess the potential application of these materials as special sorbent materials. While there is a lot of sorption data available for carbon nanotubes, little information is available on the

sorption behavior of C60 fullerenes. In a previous study we have investigated the effect of UV-induced surface oxidation on sorption behavior of C60 [1]. Changes in surface chemistry led to significant alteration in both sorption capacity and affinity. The results indicated that in addition to non-specific van-der Waals and electron donor-interactions, the presence of −OH and −COOH groups on C60 surface potentially allowed for the contribution of hydrogen-bonds. However, sorption data for a much broader set of probe compounds, covering various chemical classes, are necessary to further investigate the contribution of individual interactions to overall sorption. To systematically study this process, the combined effects of -OH/NH_2 functional groups of aromatic organic compounds and surface O-containing groups on C60 on the interactions were evaluated. We combined two multi-phase sorption batch experiment approaches using a passive sampling technique applying polyoxymethylene and a headspace in-tube microextraction. This allows for an systematic comparison of the interactions between C60 and organic compounds with significantly different physico-chemical properties (e.g., aromaticity, functional groups, etc.). [1] Hüffer T., Kah M., Hofmann T., Schmidt T.C. 2013. Environ Sci Technol 47: 6935-6942.

WE014

Immobilization of organic contaminants and heavy metals by carbonized sorbents - mechanisms and prediction

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Carbonized sorbents typically comprise only a small proportion of the soil or sediment matrix. However, they do significantly enhance sorption affinity and capacity of contaminants. Hence, carbonized sorbents are commonly used for the immobilization of both organic contaminants and heavy metals. Depending on the contaminant group, different interactions drive sorption, and different parameters may be used to predict the sorption potential of a given carbonized sorbent for a given contaminant. Therefore, the aim of this study was to identify (i) interactions that drive sorption and (ii) key parameters for the prediction of sorption for neutral organic contaminants, organic acids and heavy metals. Hence, we combined comprehensive characterization of selected carbonized sorbents with data from sorption batch experiments for selected polycyclic aromatic hydrocarbons, organic acids and heavy metals. Generally, sorption strength (K_D, kg/L) followed: polycyclic aromatic hydrocarbons > heavy metals ≥ organic acids. We found that the immobilization of neutral organic contaminants can occur via hydrophobicity driven interactions and specific interactions (i.e. π-π electron interactions and H-bonds), and depends on various factors including the grade of carbonization and availability of sorption sites. The immobilization of organic acids can additionally occur via electrostatic interactions and cation bridging and depends on factors including ionic strength and solution pH. The immobilization of heavy metals can occur via specific and non-specific electrostatic interactions, exchange, and precipitation, and depends on factors including ionic strength, pH and redox-conditions. The sorption of neutral and acidic organic contaminants can be well predicted by combining parameters commonly used to describe sorbent and sorbate properties, i.e. specific surface area, and octanol-water partitioning behavior (K_{OW} and D_{OW} for neutral and acidic compounds, respectively). For the prediction of heavy metal sorption, a case by case evaluation is needed, due to the complexity of factors influencing speciation and precipitation processes. Our findings help better predict the sorption potential of carbonized sorbents to a large range of contaminant groups. For neutral and acidic organic contaminants we present a simple assessment tool for the potential use of carbonized sorbents in environmental applications.

WE015

Vicia-micronucleus test as a newly standardized tool to assess soil genotoxicity: Application to the evaluation of the effects of biochar in contaminated soils

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The risk assessment of industrial contaminated soils is an important challenge in remediation process. Information on bioavailability of pollutants requires the development of biological tests, especially with plants. Ma (1999) described higher plants as the most sensitive organisms for the detection of mutagens and genotoxic effects of environmental pollutants. Although ecologically relevant for soil toxicity assessment, plants are surprisingly not the most commonly used organisms for genotoxicity tests (White and Claxton, 2004). This is the reason why genotoxicity tests with higher plants have been promoted (IPCS – United Nations Environment Programme, 1999). Genotoxicity - simply defined as the toxicity on the genome - is an indicator of dysfunctions appearing at sub-lethal concentrations. An easy endpoint to observe is the formation of micronuclei, that are small nuclei appearing whenever a chromosome fragment or a complete chromosome is not incorporated into the nuclei during mitosis. This endpoint is very important to include in a battery of ecotoxicity tests for a better risk assessment of contaminated soils and of the impact of remediation techniques applied to them. Among techniques of *in situ* remediation, soil amendments with biochar, *i.e.* the solid product from biomass pyrolysis, have recently been investigated for decreasing the bioavailability of metals in industrial soils. Biochar has been shown to immobilize metals both by direct sorption at its surface and by an indirect effect through an increase of soil pH (Rees et al., 2014). The effect of biochar on the actual genotoxicity potential of metal-contaminated soils is however unknown. The aim of this study was to assess the genotoxic potential of a range of soils contaminated by heavy metals (Zn, Pb, Cd) and amended by a wood-derived biochar to create a gradient of metal availability. We recently obtained international standardization of the *Vicia*-micronucleus test (ISO 29200) in 2013 and we performed it in this study by direct exposure of root tips to soils. The analysis of root morphology and root metal content completed the experiment. Results showed that these industrial soils induced genotoxicity, revealed by an increase of micronuclei frequency. Their genotoxic potential strongly decreased with the addition of biochar. Relationships between genotoxicity, soil metal extractability and root metal content will be discussed.

WE016

Modulation of metal bioavailability for two earthworm species by the use of biochar

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Anthropic activities may lead to depletion in soil organic matter contents and to soil contamination, particularly by trace elements. Biochars i.e. stable carbon-rich by-products synthesized through pyrolysis/carbonization of plant- and animal-based biomass, are used as soil amendments to improve carbon sequestration and crop yields while valorizing crop residues and stabilizing soil contaminants. The aim of this study was to assess the effect of a poultry manure-derived biochar on the bioavailability of cadmium (Cd), copper (Cu), lead (Pb) and zinc (Zn) for two earthworm species i.e. *Aporrectodea icterica* and *Aporrectodea longa* exposed in microcosms to an *in situ* contaminated soil during 28 days. For that purpose, we assessed (i) the environmental availability by measuring total concentrations and concentrations in pore water, (ii) the environmental bioavailability by measuring concentrations of trace elements in earthworms, and (iii) the toxicological bioavailability by measuring survival, weight gain, energy reserves (protein, lipid and glycogen contents) and the GST (glutathione-S-transferase) enzymatic activity of earthworms, as an indicator of detoxification processes. Contrary to what we expected, the presence of the studied poultry manure-derived biochar did not greatly influence the bioavailability of trace elements for earthworms under our conditions. We discussed assumptions to explain these results.

WE017

Modulation of trace element bioavailability, yield and seed quality of rapeseed (Brassica napus L.) by biochar addition to a contaminated technosoil

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Rapeseed (*Brassica napus* L.) is a Cd/Zn-accumulator whereas soil conditioners such as biochars may immobilize trace elements. These potentially complementary soil remediation options were trialed, singly and in combination, in a pot experiment with a metal(loid)-contaminated technosol developed on dredged sediments. The efficiency of these remediation options were assessed using both phenotypic and physiological plant responses. A trace element (Cd, Cu, Pb, Zn) contaminated technosol was either amended (2% w/w) or not with a poultry manure-derived biochar. Rapeseed was cultivated under both soil treatments during 24 weeks up to harvest in greenhouse. Based on the soil pore water, biochar incorporation into the technosol promoted the As, Cd, Cu, Mo, Ni, Pb and Zn solubility. It decreased foliar B, Cu and Mo concentrations, and Mo concentration in stems, pericarps and seeds. Conversely, it did not impact neither the biomass of aerial rapeseed parts (except a decrease for seeds), nor their C content (except a decrease for stems), seed fatty acid content, seed sugar content

and antioxidant capacity in both leaves and seeds. Biochar amendment increased the phytoextraction by aerial plant parts for K, P, and S, reduced it for N, Ca, B, Mo, Ni and Se, whereas it remained steady for Mg, Zn, Fe, Mn, Cu, Cd and Co. Zinc and Cd concentrations in the soil pore water were decreased 3 times by rapeseed in the unamended technosol showing the feasibility to strip available soil Zn and Cd in combination with seed production.

WE018

Rearing kids on chlordecone contaminated soils: Activated carbon, a promising media to limit the transfer from soil to animal tissues

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Chlordecone (Kepone) (CLD) is a highly persistent pesticide in soil which was intensively used in French West Indies to fight the banana black weevil. Because of its toxicity and considerable levels in soil, it became a major threat for human health to address . Soil or sediments are considered as reservoir for this contaminant and soil ingestion is the main exposure route for free range animals. In order to ensure the production of safe animal foodstuffs, it is necessary to reduce the transfer from soil to animal tissues. The present study intends to test whether two different activated carbons (ACs) could limit the CLD transfer from soil to animal. Three artificial soils (ASs) were prepared according to OECD guideline 207. One standard soil (SS), devoid of organic matter (OM), and two amended versions of this SS with coconut-based activated carbon (AC_1) or lignite-based one (AC_2) were prepared to obtain a 2% organic carbon content (dry matter basis). This study involved 15 kids as a typical on-farm consumption animal. Animals were randomly distributed into 3 groups (n=5 replicates). During 21 days, the kids were fed AS spiked with 10 μg of CLD per g of dry matter to achieve an exposure dose of 10 μg CLD per kg of body weight per day. After 21 days of oral exposure, CLD in peri-renal adipose tissue and liver were analysed by GC–MS, after extraction and purification. Concentrations in tissues were significantly affected by the treatment (p< 0.01). In liver, CLD concentration was 2110, 450 and 24 ng.g-1 DM for SS, AC_1 and AC_2 respectively. This decrease corresponds to a relative bioavailability of 21 and 1.2% for AC_1 and AC_2 respectively. In adipose tissue, a similar decrease has been observed. This study leads to conclude that 1/ AC could dramatically reduce the bioavailability of CLD 2/ extent of this limitation depends of the nature of AC. This result obtained with artificial soils is considered as a first major step prior to on-field experiments.

WE019

PAHs in biochars are largely desorption resistant

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When biochar is applied in agricultural practice polycyclic aromatic hydrocarbons (PAHs) that are coproduced during the feedstock pyrolysis might be released into the environment. It is therefore important to assess the exposure of the native PAHs in this carbonaceous material for humans and the living environment. We report on the PAH exposure of almost 50 different samples including biochars from different feedstocks, pyrolysed under increased oxygen content and mixtures of biochar and organic material. The total concentration ($C_{total} = C_S$) in the samples ranged from 0.4 to almost 2000 mg/kg$_{dry\ weight\ (dw)}$ and the freely dissolved concentration ($C_{free} = C_W$) from 4 to 81 ng/L for the sum (?)16 PAHs. High partition coefficients of the single PAH compounds ($K_D = C_S/C_W$ ranged from 10^4 to 10^9) lead to C_{free} of the pure biochar samples below ambient levels. These high K_Ds make a direct determination of the bioaccessibility (the concentration of the pollutant accessible for organisms over a relevant time span) difficult if not impossible, whereas desorption-resistant PAH concentrations can be determined. Therefore, a set of samples was exposed to contaminant traps in which cyclodextrin served as diffusive carrier and a composit of activated carbon and silicone as infinite sink.[1] These biochar samples were then recovered and the remaining PAHs extracted. Bioaccessibility was then calculated as concentration difference between biochars with and without exposure to the traps. Bioaccessibility of PAHs could be measured in 21% of the samples (the others showed 100% desorption resistant PAHs). The bioaccessible concentration of for instance BaP, the most carcinogenic PAH, was up to 2.8 mg/kg in a pure biochar that contained a C_{total} of 281 mg/kg$_{dw}$?16 PAHs. Nonetheless, the majority of the samples showed bioaccessible concentrations of BaP < 0.1 mg/kg. Although bioaccessibility was low in terms of numbers of observation, it was in some cases significant in terms of absolute concentrations. Biochar manufacturers are therefore encouraged to follow guidelines to minimize PAH formation during production. (1) Mayer, P.; Olsen, J. L.; Gouliarmou, V.; Hasinger, M.; Kendler, R.; Loibner, A. P. A contaminant trap as a tool for isolating and measuring the desorption resistant fraction of soil pollutants. *Environ. Sci. Technol.* **2011,** *45*, (7), 2932-2937.

WE020

Integrating analytical and effect-based biochar characterization: results from an interdisciplinary COST Action (FA TD1107) initiative

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In order to meet the proposed quality criteria and European soil and water protection targets, biochar characterization requires an integrative framework that includes analytical and effect-based approaches. Three contrasting biochars produced from mixed wood sieving (BW), paper sludge and wheat husks (BPS) and sewage sludge (BSS) according to current quality guidelines, were characterized for physical, chemical and ecotoxicological properties, in an interdisciplinary initiative of the biochar COST Action TD1107. It aimed at assessing their impact on the soil habitat function, when applied to natural (peat, Lufa 2.2) or standard (OECD soil) substrates at application rates relevant to agricultural and/or environmental management (0, 10, 50, 100, 200 t/ha). Avoidance behaviour, survival and reproduction bioassays were performed, with soil invertebrates *Eisenia andrei* (Oligochaeta) and *Folsomia candida* (Collembola), which are representative of different ecological functions. Seed germination and growth of model crop species with socio-economic relevance (*Hordeum vulgare*, *Lactuca sativa*) were also evaluated based on test procedures adapted for biochar phytotoxicity assessment. Avoidance behaviour tests showed preference by earthworms and collembolans for BW and BPS at 10 and 50 t/ha, with no preference or avoidance behaviour for BSS. There were also no significant effects on collembolan survival and reproduction (nº of produced juveniles). Plant bioassays showed that BW and BPS addition (at ca. 330 t/ha mixed to 30 cm depth) increased fresh biomass, indicating that negative effects were absent even at unusually high biochar addition rates for agricultural applications. Overall, the absence of lethal and sub-lethal detrimental effects on the test invertebrates and plants suggests a low risk to soil biota and the soil habitat function, upon amendment with the selected biochars. This result is consistent with extensive biochar analytical characterization data, as determined by state-of-the-art methodologies. We hereby demonstrated the suitability of a battery of soil ecotoxicological assays, as a complement to biochar characterization and a tool to prevent/manage possible risks to biota-mediated soil functions. It supports the prospective sustainable use of sludge-based biochars. However, longer-term impact assessments on soil biota, and the influence of biochar ageing, on its ecotoxicological potential requires further investigation.

WE021

Activated carbons prepared from plum, apricot and cherry kernels: Influence of operational parameters and elemental composition on the maximum adsorption capacity of products

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The use of alternative activated carbon derived from biomass, as agricultural waste material, has been investigated as a replacement for the current expensive methods of removing heavy metals from wastewater. The present investigation was undertaken to evaluate the adsorption effectiveness of plum, apricot and cherry kernels-based granular activated carbonsin removing Pb^{2+} ions commonly found in municipal and industrial wastewater. The primary source of biomass for the production of eco-friendly and low-cost biosorbents were lignocellulosic raw materials (fruit pits), as waste byproducts of the fruit industry and elements of organic municipal waste. Shells were activated by thermochemical conversion with phosphoric (H_3PO_4) and sulphuric (H_2SO_4) acid at 400°C and 500°C for 2 h, in the complete absence of nitrogen inert atmosphere. In the study, various parameters such as the impregnation type, activation temperature and elemental composition of kernels were evaluated to establish the separation efficiency of the Pb^{2+} ions in the optimum batch operational system mode. It was observed that theoperational parameters and composition of row materials have significant influence, in the range of 25.02 mg g^{-1} to 119.64 mg g^{-1}, on the maximum adsorption capacities of the synthesized activated carbons. The adsorbent derived from these materials is expected to be an economical product for metal ion remediation from wastewater. **Acknowledgement:** Ministry of Education, Science

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WE022

Effects of activated carbon on PCB bioaccumulation and biological responses of Chironomus riparius

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Activated carbon (AC) amendments have been studied as a new stabilizing method for contaminated sediments. The sorbtion efficiency of AC toward hydrophobic organic compounds (HOCs) has been shown in several studies. Recently the focus has been turned also to the possible secondary effects of AC amendments. At the present study the sorbtion efficiency and possible secondary effects of AC were studied with *Chironomus riparius* in a full life cycle test. Coal based AC (ø 63-200 μm) was mixed in natural sediments from PCB contaminated area. AC amendment reduced the PCB concentrations determined with both bioaccumulation test and passive samplers. Additionally the PCB concentrations of midges emerging from AC treated sediments were reduced. Adverse effects were observed on the larvae growth and development, and morphological changes were seen on the gut wall microvilli layer. On the other hand, low dose of AC (0.5% sediment dw) slightly improved reproduction, survival, larvae growth and gut wall microvilli length in one of the studied sediments, indicating that AC amendment reduced the sediment toxicity by altering the bioavailability of the contaminants. Site specific characteristics are important when the remedial measures are designed to be balance between effective contaminant bioavailability reduction and adverse effects of the amendments.

WE023

Influence of surface composition on removal of emerging contaminants from wastewater using carbonized agricultural residues

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Clean water is a necessity for life and influences health, politics and human rights. The aim of this screening study was to assess the influence of the surface composition in the adsorption capacity of carbonized materials on ten environmentally occurring and relevant water pollutants, selected for their ability to pass unreduced through STP plants. Four wastes, horse manure, tomato residues, olive pressing residues, and rice husks were carbonized using hydrothermal carbonization (HTC). HTC is a wet carbonization process conducted in water phase, under pressure and at temperatures of 220-280 °C. A 10 µg/l model wastewater containing Ochtilinone, Triclosan (biocides), Trimethoprim, Sulfamethoxasole, Ciprofloxacin (antibiotics), Diclofenac, Paracetamol, Diphenhydramine, Fluconazole (pharmaceuticals), and Bisphenol A (plasticizer) in milliQ water were used for the adsorption tests. For the adsorption test triplicates, 10 ml of the model wastewater were added to 50 mg char in 15 ml plastic tubes, which were agitated for the adsorption time (1, 3, 5, 8, 12, 18 and 25 minutes at 20 °C). The removal efficiency differed between the four biochars, with horse manure and rice husks being the most efficient, with 68% and 74% average adsorption respectively after 25 minutes. Adsorption rates were fast, with high adsorption achieved already at the first sampling time. The adsorption efficiencies correlated somewhat with the water/octanol partitioning coefficient of the substances in the model water, but not at all with the water solubility. This did not explain why the manure char was almost as good adsorbent as the rice husk char, as it only had one fourth of the surface area (4 m^2/g compared to 16 m^2/g) To further investigate the differences in adsorption capacity of the chars, the surface composition of the chars was analyzed using diffuse reflectance furrier transform infrared spectroscopy (DRIFTS) and X-ray Photoelectron Spectroscopy (XPS). The result shows a large difference in surface composition between the chars which could hold the key to the different adsorption capacities. The Identification of a low-cost feedstock that enables capture of these emerging substances could improve water treatment both in global north (as an extra step in sewage treatment plants) and global south (e.g. as rudimental water filters) to improve the human and animal welfare as well as the environmental situation.

WE024

Carbon nanotubes as an effective adsorbent for Ionic Liquids removal

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For 20 years, many scientific publications have reported the presence of new compounds in the environment – emerging pollutants (EPs). However the largest challenge in the management of EPs is identification of the chemical substances which potentially can become dangerous in the future. Therefore, in spite of the mentioned group of contaminants, another important class of pollutants can be distinguished – potential pollutants (PPs), whose occurrence in the environment has not been documented yet but such probability is high nevertheless. Due to their unique properties (e.g. thermal stability, insignificantly negligible volatility, non-flammability, miscibility with water or other solvents) ionic liquids (ILs) are videly used in in a variety of industrial processes nowadays. Therefore a danger of possible contamination of the environment by these compounds is highly probable, and hence they can be considered as PPs. The lack of fresh and clean water is an ubiquitous problem around the world. Water demand is growing rapidly as a result of increasing population and rapid urbanization. Although activated carbon (AC) is one of the most widely used adsorbents in water treatment, its application also suffers from several bottlenecks, such as slow adsorption kinetics and difficulty for regeneration. Therefore, identifying alternative materials to AC is a key issue for tertiary treatments to remove xenobiotics from WWTP effluent and further research is required with respect to the design and preparation of novel absorbents with good sorption and regeneration properties. The aim of this work was to investigate interaction mechanism between different carbon nanotubes (CNTs) and selected ILs and asses whether they may be effective adsorbents for the ILs removal from wastewater or not. The strength and extent of the sorption phenomena were determined by sorption isotherms and sorption coefficients. All the results were modelled by Freundlich and Langmuir isotherms. Obtained results show that CNTs can be considered as superior to ACs in terms of sorption kinetics. Moreover sorption of ILs on CNTs is highly effective process. The sorption mechanism has been proposed. *Acknowledgement „Program rozwoju Uniwersytetu Gdanskiego w obszarach Europa 2020 (UG2020)" project is co-financed by the European Union under the European Social Fund.*

WE025

Remediation of PCB contaminated sediments using activated carbon: Assessing efficiency and robustness using equilibrium sampling

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When released into aquatic environments, polychlorinated biphenyls (PCBs) accumulate in sediments due to their low water solubility and association with sediment organic carbon. PCB contaminated sediments have been successfully remediated using activated carbon (AC) amendments, which strongly adsorb lipophilic compounds and thereby reduce their exposure/risk to benthic and pelagic organisms. For such remediation methods, it is important to carefully assess both the method efficiency in reducing exposure and the method robustness towards ongoing influx of contamination. In the present study, a series of mesocosm experiments were performed in order to reveal how AC amendments can be most effectively applied to reduce exposure and enhance the resilience of *in situ* sediment remedies challenged by ongoing influx of contamination. In these mesocosm experiments, contaminated sediments were (i) amended with activated carbon, (ii) covered by a sand cap, (iii) covered by an AC amended sand cap, and (iv) covered by a mobile AC amended sand cap. Half of the mesocosms received ongoing influx of contamination during the 3-months experiments. Careful exposure assessments were conducted before and after treatment in order to reveal how AC amendments can be most effectively applied in sediment remediation. As part of the exposure assessments, equilibrium sampling of the sediments were used to determine: (1) the freely dissolved PCB concentrations in sediment pore water (C_{free}, pmol/L), (2) the PCB chemical activities in sediment (a, unitless), and (3) the PCB concentrations in lipid at thermodynamic equilibrium with sediment ($C_{lipid \leftrightarrow sediment}$, mmol/kg). The freely dissolved concentration is frequently perceived as the effective concentration for bioconcentration and toxicity, while chemical activity (a medium-independent exposure parameter that quantifies the energetic level, and not the concentration, of an organic compound relative to its energetic level in pure liquid [0-1, unitless]) governs diffusion and equilibrium partitioning. Both freely dissolved concentrations and chemical activities express the effective exposure as opposed to the more traditionally used measure of total concentration. Still, the main novelty of this approach is that $C_{lipid \leftrightarrow sediment}$ can be determined in a practical and precise manner to express the thermodynamic potential for bioaccumulation (i.e. the concentration in the lipid of an organism at thermodynamic equilibrium with sediment).

WE026

Effects of biochar on the dynamic of metals in soils polluted by mining wastes under changing hydric conditions

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The aim of the present study was to assess the effects of biochar and the presence/absence of vegetation on the fate of metals in wetland soils polluted by metal mining wastes to ascertain the effectiveness of these techniques on the improvment of soil conditions and the reduction of the environmental risks associated to metal pollution. A column (15 x 30 cm) experiment with two types of soils (one with pH~4.7 and another one with pH~7.4), two types of biochar (one from municipal solid wastes -MSW- and another one from sewage sludge -SS-) and with the presence/absence of plants (halophyte species *Sarcocornia fruticosa*) was performed with alternating flooding-drying conditions inside a greenhouse. The water level was kept at 5 cm above the soil surface for one month and at 15 cm below the soil surface for the following month. This cycle was repeated several times along the experiment. The pH and redox potential (Eh) were regularly checked as well as the dissolved organic carbon (DOC) and soluble metal concentrations (Cd, Mn, Pn and Zn) in pore water. In the basic soil the biochar application did not have any effect on soil pH. In the acidic soil the MSW biochar increased the pH from ~4.5 to 6.0-6.5 while the SS biochar to ~5.5. The presence of vegetation did not have any effect on soil pH. The SS biochar greatly decreased the Eh values in both study soils due to a higher input of DOC and then probably to a greater microbial activity. In the acidic soil the higher pH due to biochar application led to a decrease in the soluble metal concentrations regardless the Eh values and the presence/absence of plants. In the basic soil the drop of Eh values due to the flooding conditions favoured higher soluble Mn and Zn concentrations probably due to the solubilisation of metal oxides. The MSW biochar favoured higher soluble Cd concentrations after one month of flooding although the concentrations decreased when decreasing the water level. The dynamic of Pb was similar among all the treatments assayed, regardless the biochar and the presence/absence of plants, and was conditioned by the flooding regime. The Pb concentrations in pore water increased during the flooding periods and decreased during the drying ones probably due to the metal immobilisation/mobilisation in relation to the dynamic of sulfurs. The effectiveness of the treatments assayed (biochar application) depended on the soil type, the hydric regime and the presence/absence of vegetation.

Passive sampling of organic micropollutants and toxicity assessment: opportunities, challenges and innovations (P)

WE027

Bioaccumulation of hydrophobic organic contaminants in mussels in the Istanbul Strait: Field monitoring by means of passive water sampling and modelling

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Determination of the concentrations of hydrophobic organic contaminants (HOCs) such as PAHs (polycyclic aromatic hydrocarbons) and PCBs (polychlorinated biphenyls) from water samples is difficult due to the low water solubility, high lipophilicity, strong adsorption tendency to suspended materials and tendency to accumulate in organisms. Biomonitoring and passive sampling methods are alternative ways of assessing the sources and fate of HOCs in the marine environment. Bivalve molluscs and Semi-Permeable Membrane Devices (SPMDs) are widely used in monitoring studies to collect information about water concentrations of HOCs. The simultaneous consideration of concentration data of bivalves and SPMDs enables conclusions about accumulation of HOCs in bivalves, even without information about water concentrations, because of their similar uptake processes. In this study, field monitoring data from the Istanbul Straitispresented. The Istanbul Strait is a busy route for both national and international navigation and hence has high potential for contamination. Specimens of the Mediterranean mussel species *Mytilus galloprovincialis* were deployed and stayed together with SPMDs in the water column for up to 21 days. A published model was adapted and used to calculate depuration rate constants and bioaccumulation factors for the mussel and a series of PAH and PCBs. Model interpolations based on separate PAH and PCB data match the trend in the data well, model interpolation using a hyperbolic fit of BAF factors show an inverse trend as compared to the single-substance models. Elimination rate constants are decreasing with Log K_{OW}>6 values and can be fitted best by a hyperbolic function for the combined PAH and PCB data. In conclusion, bioaccumulation and depuration kinetics of HOCs in *M. galloprovincialis* can be modelled properly based on Log $K_{OW.}$ While depuration constants show a clear hyperbolic trend, for the bioaccumulation factor's dependency on Log K_{OW},it is notcompletely clear whether linear or hyperbolic regression fits best. Using the hyperbolic regression equation in interpolation of SPMD/mussel concentration ratios (C_S/C_M), results in a completely switched response of C_S/C_M ratios for changing Log K_{OW}. Inclusion of more HOCs in monitoring studies will help to further investigation of this relation. Differences in the C_S/C_M ratios for different exposure durations can be modelled, and the duration of future monitoring experiments can be decided effectively.

WE028

Calibration of Polar Organic Chemical Integrative Sampler (POCIS) in wastewater treatment plant, and pesticide monitoring in wastewater network

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Because of generalized uses of pesticides by agriculture, private consumers, industries or public authorities, pesticides are released into the environment, potentially affecting ecosystems. Reduction at source can be considered for some compounds. Wastewater via wastewater treatment plants (WWTP) have shown to be an important source of pesticides to the environment. Wastewaters are specific matrices, difficult to monitor and heterogeneous, with high amount of organic matter, variable water depths and high variability of pesticide concentrations. All these drawbacks can compromise the regular sampling of wastewater, so passive sampling seems to be a good alternative to high frequency grab sampling for pesticide monitoring. This study evaluated the potential of Polar Organic Chemical Integrative Sampler (POCIS) as medium term samplers. POCIS were calibrated *in-situ*, in a WWTP influent, and on a small peri-urban river, generating sampling rates for a large amount of pesticides. Some POCIS were also deployed on wastewater network and in the river for short term monitoring of pesticides, conducing to calculation of time weighted average concentrations. These experiments brought to light that the time of exposure in wastewater was short (about 3 days) for a quantitative purpose. Moreover the devices appeared to be not so appropriate in such environments due to solids that have been accumulated on them, and could have led to wrong estimation of time weighted average concentrations in wastewater. New designs of the sampler were tested in lab and on field to improve the sampling capacity of this dispositive. A better knowledge of the contamination of micropollutants in wastewater can lead to the identification of sources in wastewater network, reducing contamination at source, in the perspective of protecting water resources. *Acknowledgement* - This study benefited from funds in relation with RESEAU and "Plan Micropolluants" projects (associating as partners: Bordeaux Métropole, SUEZ, IRSTEA, Water Agency, CNRS, University of Bordeaux, CHU and Aquitaine Regional Council). This work was funded by the Aquitaine Regional Council and SUEZ. This study has been carried out with financial support from the French National Research Agency (ANR) in the frame on the Investments for the future programme, within the Cluster of Excellence COTE (ANR-10-LABX-45)

WE029

Effects of Flow Conditions on POCIS Sampling Rate for Constant Pesticides Concentration Exposures

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The effects of hydrodynamic conditions on polar organic chemical integrative sampler (POCIS) uptake rates for a range of pesticides were investigated in a controlled laboratory setting. The POCIS devices were exposed to constant concentrations of pesticides under different flow conditions (static, 1 $cm.s^{-1}$, 3 $cm.s^{-1}$, 4 $cm.s^{-1}$ and 5 $cm.s^{-1}$) over a period of 20 days. The results showed that for most compounds, there was an increase (up to more than one order of magnitude) of sampling rate with increasing flow velocity. However, this trend was slightly different for the neonicotinoid thiamethoxam, where a lower sampling rate was observed at 5 $cm.s^{-1}$ compared to flow conditions of 1, 3 and 4 $cm.s^{-1}$. Generally, a positive correlation was observed between the sampling rates and hydrophobicity (log octanol/water partition coefficient, log K_{OW}) for pesticides with log K_{OW} ranging from 1 to 3. Study results also include the first reported estimates of POCIS sampling rates for bicyclopyrone and cyproconazole.

WE030

Passive sampling: A viable technique for post registration monitoring? A review.

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Passive sampling techniques have been developed and successfully deployed for monitoring pesticides in surface, ground and marine waters, as well as air sampling and sampling for bystander exposure. To date, passive sampling has not been recognised as a method suitable for data collection within regulatory studies, possibly due to uncertainties involved with the extensive calibration procedures and matrix selection usually required. However, with the extended provision for post registration monitoring of pesticides under the current pesticide Regulation (Regulation (EC) No. 1107/2009), passive sampling may provide a cost effective alternative to conventional sampling techniques. Although no specific guidance on the use of passive samplers for pesticide monitoring in the regulatory context currently exists under Regulation (EC) No. 1107/2009, use is mentioned in Directive 2013/39/EU "Priority Substances", stating; "novel monitoring methods such as passive sampling and other tools show promise for future application, and their development should therefore be pursued". Additionally, although not directly referred to in Directive 2000/60/EC "The Water Framework Directive", passive sampling is listed as a complementary sampling method within the WFD guidance document, stating that "passive sampling can be used alongside spot sampling to confirm or refute the results of spot sampling". This review will consider current developments in passive sampling technology in relation to monitoring pesticides, and their relevance in relation to data submission for regulatory requirements.

WE031

Pesticide quantification in POCIS extracts: matrix effect due to polymers in polyethersulfone membranes and washing protocol

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Passive samplers are exposed in the aquatic environment for some days and a time-weighted average concentration (TWAC) can be calculated which improves the representativeness of pollutant concentration, compared to punctual sampling. For pesticide residues, the Polar Organic Chemical Integrative Sampler (POCIS) prepared from a sorbent material (Oasis HLB) enclosed between two polyethersulfone (PES) membranes can be used. To mitigate the effects of environmental parameters (fouling, variations in flow velocity), performance and reference compounds (PRC) are applied. The presence of polyethylene glycol compounds (PEG) released by PES membranes in POCIS extracts was shown by high resolution time-of-flight mass spectrometry. PEG compounds induce strong matrix effects during PRC and target molecule quantification. Dilution of POCIS extracts can reduce this matrix effect but decreases POCIS performance (quantification limit). As confidence in quantification is the key to a satisfactory interpretation of environmental contamination, this work aimed at investigating the effect of analytical interferences on pesticide and PRC quantification and also proposing a washing protocol for PES membranes [1]. To study interferences, a matrix collected from POCIS exposed in freshwater was used. POCIS extracts were diluted at different levels (1/10; 1/25) and spiked with a pesticide mixture (from 10 $\mu g.L^{-1}$ to 100 $\mu g.L^{-1}$). A suppression of DIA-d5 signal (deisopropylatrazine) was induced by interferences. As this substance is used as a PRC, the quantification bias hamper the TWACs calculated for the other pesticides. A washing procedure consisting of two successive baths with ultra pure water (UPW) / methanol (50/50) and a final rinse with UPW was proposed to limit PEG release. Total ion current chromatograms of extracts from POCIS built with washed PES membranes did not display a significant PEG fingerprint. The removal of PEG interference allows proper quantification of DIA-d5 and pesticides during the PEG retention time range (3.5 – 5.5 min) with a 10-time dilution. With a slight extract dilution (10 instead of 25 times for POCIS with unwashed membranes), pesticides at very low concentration can be properly detected and quantified which further improves the ability of POCIS to quantify molecules at very low content in water, particularly those eluting with PEG contaminants. [1] R. Guibal, S. Lissalde, A. Charriau, G. Guibaud. 2015. Talanta, 144:1316-1323.

WE032

Sorption of structurally different ionized pharmaceutical and illicit drugs to a mixed-mode coated microsampler

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The mixed-mode (C18/strong cation exchange-SCX) solid-phase microextraction (SPME) fiber has increased sensitivity for ionic compounds compared to more conventional sampler coatings such as PDMS and polyacrylate. However, data for structurally diverse compounds to this sampler coating are too limited to define structural limitations of this type of passive sampler. We determined C18/SCX fiber partitioning coefficients of 17 cationic structures without hydrogen bonding capacity besides the charged group, stretching over a wide hydrophobicity range, and 8 strongly basic pharmaceutical and illicit drugs with additional hydrogen bonding moieties. In addition, several neutral benzodiazepines and the acid diclofenac were tested. All tested compounds showed nonlinear isotherms above 1 mmol/L coating, and linear isotherms below 1 mmol/L. The affinity for C18/SCX-SPME for tested organic cations without H-bond capacities increased with longer alkyl chains, ranging from log$D_{\text{fiber-water}}$ 1.8 (benzylamine) and 5.8 (triflupromazine). Smaller amines than benzylamine may thus have limited detection levels, and cationic surfactants with alkyl chain lengths >12 carbon atoms may sorb too strong to the C18-SCX sampler which hampers calibration of the fiber-water relationship. The logD_{fw} for these simple cation structures closely correlates with molecular volume and the octanol-water partition coefficient of the neutral form ($K_{\text{OW,N}}$). Oxygen moieties in organic cations decreased the affinity to C18/SCX-SPME, in comparison to the relationship with molecular volume established with the simple cationic structures, within a factor of 3 of the $K_{\text{OW,N}}$ relationship for simple cations, and only for 2/8 of these polar cations to a more than 10 times lesser extent than predicted by $K_{\text{OW,N}}$. Neutral benzodiazepines sorbed a log unit stronger than their $K_{\text{OW,N}}$. Results for anionic diclofenac species ($K_{\text{OW,N}}$ 4.5, pKa 4.0, log$D_{\text{fw,pH7.4}}$ 2.9) indicate that C18-SCX, despite the negatively charged SCX moieties, may even be useful for sampling a wide range of other dissociated carboxylic acid structures (surfactants, pharmaceuticals) and other types of organic anions (sulfates, sulfonates) that are notoriously difficult to sample with bulk polymer phase coated samplers. This data demonstrates the applicability of C18-based SPME in the measurement of freely dissolved concentrations of a wide range of ionizable compounds in many types of environmental and pharmacological studies.

WE033

Equilibrium sampling in a semi in situ pot experiment to measure freely dissolved concentrations of hydrophobic organic compounds.

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Progress has been made to develop and apply *in situ* passive sampling techniques for sensing hydrophobic organic contaminants (HOC) in the field, mainly in air, water and sediments. However, there is very little information on the capability of these methods to assess the bioavailability of HOC in soil. Under field conditions, environmental factors such as temperature, ionic strength or water content may influence the phase distribution of HOCs. Conducting passive sampling of HOCs *in situ* has the advantage of taking these actual environmental conditions automatically into account, in contrast to standard laboratory tests, which are performed under saturated water conditions (i.e. in suspensions). Here, we present an *in situ* pot experiment to assess bioavailability of polycyclic aromatic hydrocarbons (PAHs) in a historically contaminated soil with two different passive sampling techniques: low density polyethylene (LDPE) [1] and polydimethylsiloxane (PDMS) glass fibers [2]. The objective of this study is to investigate the concordance of the passive samplers, with the more widely used *ex situ* laboratory method using soil suspensions. Soil was excavated from the upper 20cm of a pigeon shooting range in Embrach, Switzerland, which was contaminated with 270 mg/kg total concentration of the sum of the 16EPA PAHs. The soil was thoroughly mixed according to Bucheli et al. [3], and the samplers were deployed directly into the pots with 1.5 kg soil and put into the greenhouse. The samplers were exposed for 3, 6 and 9 months. Some pots were kept at 60% water saturation, which is close to field capacity, and others at full 100% saturation. Results of the two passive samplers from both experiments will be presented and correlated to each other, and the outcomes will be compared to PAH bioaccumulation by earthworms and plants also derived from the very same pot experiment. This research will help to evaluate the applicability of these passive samplers directly *in situ* under soil unsaturated field conditions.

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WE034

Soil-atmosphere exchange of persistent organic pollutants: Determination of concentration gradients with passive samplers

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Since the last century soils have accumulated plenty of anthropogenic chemicals like the persistent organic pollutants (POPs) which might revolatilize due to decreasing atmospheric concentrations during recent decades. As a contaminant class within the group of POPs the polycyclic aromatic hydrocarbons (PAHs) feature characteristic properties as probable human carcinogens with relatively high concentrations in surface soils, allowing accurate measurements of concentration gradients, and are therefore a relevant class of chemicals of concern. The direction and magnitude of diffusive contaminant flux across the soil–air interface is controlled by the gradient of gas-phase concentration. Past investigations into the soil–air exchange of contaminants have relied on estimation methods for calculating contaminant fugacity in soil because appropriate methodologies for measuring these gradients are not available. This study aims to overcome these problems by using time-integrating passive samplers to determine concentration gradients in the soil and across the soil–atmosphere interface. Passive samplers circumvent the need of estimating a contaminant's soil partitioning properties by acting directly as a sensor for contaminant activity (e.g. fugacity or availability) in environmental media. 80 μm thick low-density Polyethylene (LDPE) sheets spiked with performance reference compounds (PRC) were deployed in the field to sample atmospheric concentrations of PAHs. Deployments during summer, winter and spring were conducted to check on seasonal variations and correlations with climatic conditions. By comparing PAH concentrations within covered and uncovered sheets the impact of photodegradation on the accumulated PAHs was tested, which can be considered exclusively significant with respect to the PRC and depending on the season. Soil samples were taken at 5 intervals, up to 50 cm depths and equilibrated ex-situ with 30 μm thick LDPE sheets. Evaluation of concentration gradients in the soil, sorption/desorption behaviour of PAHs, as well as soil characteristics such as soil texture, porosity, water content and amount and type of organic matter was performed to verify further correlations with the exchange at the soil-atmosphere surface. The monitoring outcomes from the field and laboratory studies are used in the validation of a numerical model of soil-atmosphere exchange of persistent organic pollutants.

WE035
Calibration of PDMS and XAD-coated PDMS for measuring organophosphate flame-retardants (OPFRs) and perfluorinated compounds indoors
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Passive air samplers (PAS) are increasingly employed for monitoring semi-volatile organic compounds (SVOC) concentrations in the environment, although less work has been done on their use indoors. We report on passive air samplers consisting of polydimethylsiloxane (PDMS) and a newly designed PDMS coated with styrene divinyl benzene co-polymer (PDMS-XAD), in comparison to the popular polyurethane foam (PUF) with a single bowl shelter. An indoor air calibration study was conducted to determine passive sampling rates by deploying these PAS and comparing with air concentrations measured using two low-volume active air samplers analyzed for gas and particle phases separately. Analytes of interest are brominated and organophosphate flame retardants, phthalate esters and polycyclic aromatic hydrocarbons (PAHs) and perflourinated compounds. The study extended for 50 days. Surface-area normalized uptake rates of PDMS were comparable to PUF using the single bowl shelter, ranging between 0.6 to 1.5 m^3 day^{-1} dm^{-2} for brominated flame retardants and phthalates. Preliminary results showed that the number of target chemicals was increased with the PDMS-XAD design because of differences in chemical properties of PDMS and XAD.

WE036
Estimating Uncertainties in Air-Water Diffusive Exchange Flux and Gross Volatilization Loss of PCBs: a Case Study based on Passive Sampling in the Lower Great Lakes
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Air-water diffusive exchange is a significant process in PCB geochemical cycling across the Great Lakes. Compared with dry and wet deposition fluxes, the diffusive flux cannot be directly measured experimentally. Its model-based calculation contained considerable uncertainty because of uncertainties in atmospheric and aqueous concentrations and meteorological conditions. To capture inherent variability of the diffusive flux and load of PCBs, 57 pairs of air and water samples from 19 sites covering the Lake Erie and Ontario were collected using passive sampling during 2011-2012. Error propagation analysis and Monte Carlo simulation (MCS) were applied to estimate uncertainty in the diffusive flux. Passive sampling technique can provide a more precise estimate on direction of air-water diffusive exchange of PCBs. Best-fit values of wind speed and temperature from probability distribution function can quantify more precisely the site-specific diffusive flux. We confirmed that 30-40% of relative uncertainty in overall mass transfer velocity was a reasonable and acceptable assumption in error propagation to a large extent. Results from error propagation analysis were comparable with those from MC simulation, but the former would not be recommended in net deposition situation. Parameters sensitivities were different in net deposition, volatilization and equilibrium situations. Volatilization flux of 7 PCBs with maximum frequency across Lake Erie and Ontario was 618 pg m^{-2} day^{-1} and 426 pg m^{-2} day^{-1}, respectively, and annual gross volatilization loss of 7 PCBs was 45.65 kg $year^{-1}$ for Lake Erie and 32.42 kg $year^{-1}$ for Lake Ontario in 2011-2012.

WE037
Persistent organic pollutants (POPs) at Ross Sea (Antarctica) and through Circumpolar Deep Water.
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The Antarctic region is ideal place for environmental studies of the marine ecosystem both near the coastal line and off-shore, due to the almost total absence of local pollution sources. In fact, pollutants reach Antarctica almost exclusively by long range transport processes involving both the atmosphere and the hydrosphere. The Circumpolar Deep Water (CDW), the largest circulation feature of the Southern Ocean, is mainly responsible for the rather limited exchange processes between the Antarctic seas and the outer oceans, thus it may be a possible source of persistent organic pollutants (POPs). The most significant findings on the presence of POPs in the marine ecosystem at Ross Sea and on CDW circulation will be discussed. Seawater samples were collected in many sampling sites located in a large area of the Ross Sea and during the travel to and from Antarctica during the XXVIIth Italian expedition. Two classes of POPs are considered, namely polychlorobiphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs). Some data was also obtained on the mixing process between two important water masses present in the area of the Ross Sea under investigation: the Modified Circumpolar Deep Water (MCDW) and the High Salinity Shelf Water (HSSW). The MCDW, relatively warmer, comes from the external oceanic circulation in the Pacific sector of the Southern Ocean, whereas the HSSW, relatively colder, is generated inside the Ross Sea basin. Both move towards Cape Adere where they mix. The intrusion of MCDW in the colder HSSW is clearly evident: the very sharp change in the temperature at about 170m depth from -1.5 ºC (typical of the HSSW) to +0.2 ºC (typical of the MCDW) is in very good agreement with a sudden change in the PCB concentration by a factor of two. The same behaviour was also observed for the total content of PAHs. This is the first experimental evidence of the pollutant input in the Ross Sea basin from the external oceanic circulation, which happens in a period of the season when the ecosystem is particularly sensitive since the biological activity is at its peak.

WE038
Laboratory calibration of two passive samplers for aquatic organic contaminants: POCIS and a new mixed polymer sampler
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Uncertainty in the determination of the compound-specific sampling rates is a major issue leading to errors in the conversion of Polar Organic Chemical Integrative Sampler (POCIS) measurements into environmental dissolved concentration, and is one of the main challenges with kinetic passive sampling. The affinity of compound to the sorbent and membrane used for constructing POCIS, as well as the sampling rate, are all compound dependent. Therefore, it is important to better understand the uptake kinetics of such passive samplers aimed at sampling polar organic compounds. Furthermore, relating these uptake kinetics to the compound properties will allow the sampling rates of other compounds to be estimated when measurements are not available. Polydimethylsiloxane (PDMS) silicone is commonly used for the passive sampling of neutral hydrophobic organic compounds in aquatic environments. PDMS passive samplers are directly exposed to the surrounding aquatic environment, which maximizes accumulation but also simplifies the uptake process. Other advantages include reversible sorption, which is relevant when considering the application in passive dosing mode for toxicity test. Unfortunately, PDMS is not an ideal choice for polar organic compounds because of its lower partitioning affinity which leads to problems with analytical detection. The above issues raise the question whether it is possible to combine the advantageous properties of PDMS sampler with the higher affinity of a sorbent such as Oasis HLB to make a sampler for both polar and non-polar compounds. In this study, Oasis HLB were embedded within PDMS disks to make a mixed polymer sampler (MPS). MPS disks had a high affinity for both polar and non-polar hydrophobic organics and were much easier to handle since they consisted of a single polymer block. The uptake kinetics of a range of compounds from the aqueous phase to the MPS and the traditional POCIS were compared using a batch set-up. The sampling rate to each sampler was calculated by fitting a numerical model. Subsequently, the desorption of the compounds from the equilibrated samplers was measured. These measured uptake and desorption kinetics were related to the physico-chemical properties of the chemicals as well as the sampler architectures. Therefore, the MPS had a similar performance to the traditional POCIS which the advantage that it was easier to handle and can potentially be used for passive dosing in toxicity testing.

WE039
Investigating methods for in tissue equilibrium passive sampling of POPs in lean fish
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Estimation of bioaccumulation factors (BAF) of hydrophobic substances requires measurement in aquatic biota as well as in the water phase at a steady state. Passive sampling is used extensively to quantify freely dissolved concentrations of persistent organic pollutants (POPs) in sediment pore water and surface water as a relevant parameter for the assessment of organism exposure. Furthermore, passive sampling can also be applied in fish tissue. Passive samplers that are equilibrated with water, sediment and fish would contain equal POP concentrations if the fish was at the state of a thermodynamic equilibrium with water and/or sediment. If concentrations were not equal the degree of disequilibrium would be revealed, which is equally important for bioaccumulation studies. The presently applied method for in tissue passive sampling is restricted to application for lipid rich fish tissues since the uptake capacity in lean fish of the sampler may easily exhaust the POPs from the tissue in contact with the sampler. That ultimately leads to an underestimation of the POPs concentration level in the tested tissue. We investigated several ways to solve this problem using specimen of lean fish (e.g. pike, *Esox lucius*) and more lipid-rich fish such as carp (*Cyprinus sp.*).

Equilibrium partitioning of PCBs and PBDEs between tissue and silicone rubber samplers was investigated by daily moving samplers to another position in the tissue to avoid depletion. Sampler movement was performed manually and done 4 times (4 days exposure in total). A parallel experiment was performed by rolling pieces of fish tissue together with passive samplers in a glass jar for 4 days. All incubations were done at 4°C. In all experiments the degree of equilibrium was monitored by quantifying the release of performance reference compounds (PRCs) dosed to the sampler prior to exposure. Data were compared with measurement of total POP concentration of analytes in tissue samples. Derived method figures of merit include the minimum exposure time to reach partition equilibrium for all investigated substances, its relationship to tissue lipid content, as well as a minimum mass of tissue required to reach quantification limits. This method has a large application potential for bioaccumulation studies, also for some emerging environmental contaminants.

WE040

Quantifying the effects of temperature and salinity on partitioning of hydrophobic organic chemicals to silicone rubber passive samplers

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Nowadays, passive sampling is a widely applied technique to determine freely dissolved aqueous concentrations of hydrophobic organic chemicals (HOCs), such as polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs). Crucial to the measurements are sampler-water partition coefficients, which are generally determined in the laboratory under 'standard conditions' (in freshwater at 20 °C). Theoretically, however, the coefficients are dependent on environmental conditions, such as temperature and salinity. Yet, there are insufficient experimental data in the scientific literature to prove this for different polymers. Several polymers are already being applied during field monitoring however, and neglecting any effects may lead to imprecise results. In the present study, we therefore quantified the effects of temperature and salinity on the sampler-water partition coefficients of PAHs and PCBs for silicone rubber, a material used in Dutch passive sampling monitoring campaigns. The results demonstrated a chemical-specific and hydrophobicity-dependent temperature effect, being independent of salinity; and a chemical- and temperature-independent salinity effect. Based on the obtained data, location-specific silicone rubber-water partition coefficients ($K_{sr\text{-}w}$; adjusted for temperature and salinity) can be calculated. The impact of applying such location-specific values was demonstrated using the Dutch passive sampling field monitoring database, covering ten-years of PAH and PCB data for several locations. Adjusting the $K_{sr\text{-}w}$ values resulted in aqueous concentrations that were lowered by a factor of 1.6 on average. The reduction was rather constant because of the manner of sampling (under non-equilibrium conditions and using performance reference compounds) and calculating. When sampling under equilibrium conditions in seawater at temperatures at about freezing, and/or applying different calculation approaches, the adjustment effect can potentially increase up to a factor of about 5-6 for the more hydrophobic PAHs and PCBs. Although this study exclusively focused on silicone rubber, qualitatively the results will also apply to other passive sampling materials.

WE041

Combining passive sampling and 'slow stirring' to determine octanol-water partition coefficients for extremely hydrophobic chemicals

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Octanol-water partition coefficients (K_{ow}) are widely used in fate and effects modelling of chemicals. Still, high quality experimental K_{ow} data are scarce, in particular for very hydrophobic chemicals. This hampers reliable assessments of several fate and effect parameters and the development and validation of new models. One reason for the limited availability of experimental values may relate to the challenging nature of K_{ow} measurements. In the present study, K_{ow} values for 13 polycyclic aromatic hydrocarbons (PAHs) were determined with the gold standard 'slow stirring' method ($\log K_{ow}$ 4.6-7.2). These values were then used as reference data for the development of an alternative method for measuring K_{ow}. This approach combined slow stirring and equilibrium sampling of the extremely low aqueous concentrations with polydimethylsiloxane-coated solid phase micro extraction (SPME) fibers, applying experimentally-determined fiber-water partition coefficients. It resulted in K_{ow} values matching the slow stirring data very well. Therefore, the method was subsequently applied to a series of 17 moderately to extremely hydrophobic petrochemical compounds. The obtained K_{ow} values spanned almost 6 orders of magnitude, with the highest value measuring $10^{10.6}$. The present study thereby demonstrates that the hydrophobicity domain within which experimental K_{ow} measurements are possible can be extended with the help of SPME; and that experimentally-determined K_{ow} values can exceed the proposed upper limit of 10^9.

WE042

Passive sampling strategies to monitor Polycyclic Aromatic Hydrocarbon (PAH) emissions to rivers during base flow and flood waves

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Polycyclic aromatic hydrocarbons (PAH) remain an issue in the WFD due to very low EQS for higher molecular compounds of this group. Sources of PAH are often manifold reaching from street runoff to alluvial groundwater inputs from contaminated sites and the pollution pathways are dynamic. This confronts water managers with enormous challenges in investigative monitoring. We explored the use of two types of passives samplers to investigate the flow pathways and phase in which PAH are transported: Sediment nets are continuously collecting suspended matter in rivers and are low-tech enough to be able to perform longitudinal profiles with multiple sampling points in order to locate PAH sources. In the same time Empore discs are used to collect dissolved PAH from the water phase. The hypothesis was that – according to the source – in variable hydrological situations and source locations differences would be expected between particulate and dissolved collected PAH depending on pathway and freshness of the contamination. In order to test this hypothesis 3 locations with different sources in a longitudinal profile on a river have been chosen: 1) a site with urban emissions (combined sewer overflows) 2) a site with a tributary input from a large gas station 3) a site with a tributary with a WWTP input. Results showed that large hydrological events were dominated by erosion with background contamination of PAH and identical levels and relationships of particulate vs dissolved concentrations in the three sites. During low flow and smaller events differences between the sites were obvious especially in the Empore disc collected phase, showing the presence of freshly contaminated suspended matter (rapidly desorbing) or alluvial groundwater inputs at the gas station site. In general the apparent Koc for individual PAH calculated from suspended matter contents and time weighted average concentrations from Empore discs were two orders of magnitudes above he equilibrium Koc from the literature. This shows that pollution of particulate matter in rivers is often aged and equilibrium calculations for EQS evaluation can introduce great bias.

WE043

Evaluating pharmaceuticals in rivers as tracers for WWTP performance in whole catchments

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Pharmaceuticals display variable fate in waste water treatment, but also following emission to rivers. Some compounds like carbamazepine can be used as a conservative tracer and can be directly related to population equivalents emitted in a river catchment. Other compounds reflect the performance of treatment plants and their presence in rivers can be used to evaluate the overall degradation efficiency in a river catchment. This requires the in-river fate of the compounds to be quantifiable: Indeed some compounds are subject to photodegradation or microbial transformation during transport in a river. Here we investigate the fate of a dozen pharmaceuticals using 1) diel autosampler campaigns to evaluate photochemical degradation and 2) longitudinal profiles with passive samplers and grab samples to evaluate overall mitigation over a stretch of 12 km downstream a major waste water treatment plant in Luxembourg. Grab sample profiles are prone to errors due to potential pollution peaks whose travel time cannot be considered in longitudinal profiles. Passive samplers overcome this problem by averaging over 14 days. In addition the comparison of passive sampling and grab sampling longitudinal profiles provides insight in the mitigation process: grab samples reflect mostly day-time processes while passive samplers cover the night period too. Hence, different mitigation rates indicate the photodegradation to be relevant. Three campaigns in series have been conducted and confirmed the feasibility of the concept although non-constant performance of the WWTP and the mid-term occurrence of a sewer discharge in the longitudinal profile reduced the pertinence of the data.

WE044

Application of passive sampling technique for monitoring 64 pesticides in Japanese surface water

Y. Kameda, Chiba Institute of Technology / Civil Architectural and Environmental Engineering

To monitor various pesticides in surface water is very important in an aquatic environment. Especially, continuous or frequent monitoring is desirable in the source of water supply. In Japan, there are several hundred species of pesticides to monitor in surface river water as well as purified water in order to supply "safe" drinking water. However, not all the pesticides are investigated at most rivers and lakes for drinking water. Furthermore, their water samples are generally collected once a month though a large amount of herbicides are spread over paddy fields in every spring and summer. Therefore, novel monitoring techniques are needed for simultaneous analysis and continuous monitoring for a variety of pesticides in surface water. Passive sampling technique is one of the possible monitoring methods, which can estimate time-weighted average concentrations (TWA) and can accumulate concentration data during sampler deployment. In this study, passive sampling technique was developed to monitor 64 pesticides mainly used for paddy field. Empore SDB-XD disks were used for absorbent and put them into Chemcatcher® holders. Calibration experiments using flow-through exposure systems were conducted in order to reveal sampling rates for 64 pesticides in our laboratory. Absorbed mass of 64 pesticides were analyzed by GC/MS. The calibration tests were performed under four different conditions of water velocity. Some chemicals like isotopically aromatic hydrocarbons were added as performance reference compounds and flow monitors. We also applied this passive sampling technique to some rivers and revealed seasonal trends of 64 pesticides TWA and compared TWA and grab sampling data.

WE045

Developing ceramic passive samplers to monitor cytostatic drugs in river water

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Cytostatic drugs have recently been detected in river waters as a result of its high and increasing consumption, becoming new water contaminants. Currently, grab sampling is the most common way to collect samples from rivers although it might not be completely representative of the river quality due to time dependant variability. In a previous study, grab sampling monitoring was performed in a drinking water treatment plant (DWTP) and along the Besòs River (near Barcelona, Catalonia, NE Spain). In the DWTP intake, mycophenolic acid was detected at concentrations between 17 to 52 ng L^{-1}. In the Besòs River, two sampling campaigns were performed and different concentration profiles were obtained along the river for 14 cytostatic drugs, showing that their emission to the waters is reiterative but with high concentration variability. Therefore, passive samplers can be used to better evaluate the presence of cytostatic drugs in the aquatic environment, as they can be efficient, relative inexpensive and provide time integrated concentrations. Ceramic passive samplers had been developed, using porous ceramic tubes (45 mm x 13 mm, ID 9 mm) and 200 mg Zepra ZT (30 μm, 85 Å, Phenomenex) adsorbent. The adsorbent was conditioned with MeOH and water, and the tubes were filled. If needed, more water was added to completely fill the internal volume in order to facilitate the drugs diffusion. Then, they were kept in milli-Q water until deployment. For laboratory test, 10 devices were immersed in a 4L solution containing a mixture of 16 cytostatic drugs at 20 μg L^{-1}. They were deployed from 2 to 11 days to evaluate their adsorption, and two samplers were removed every 2 days. The adsorbent was removed from the tube and was extracted using MeOH in an ultrasonic bath. Linear adsorption was observed for most of the drugs, being up to 200 ng after 11 days, which allowed calculating the diffusion coefficients and the sampling rates. Then, 6 devices were deployed in the DWTP intake during 1 to 3 weeks to see the fouling effect. Finally, the passive samplers were deployed in the DWTP intake and in the Besòs River.

WE046

Passive sampling technique combined with ELISA assay detection for monitoring neonicotinoid insecticides in Japanese surface water

Y. Kameda, Chiba Institute of Technology / Civil Architectural and Environmental Engineering

Neonicotinoid insecticides are widely used in Japan. Especially, seven neonicotinoid insecticides such as Imidacloprid, Acetamiprid, Thiocloprid, Clothianidin, Dinotefuran, Thiamethoxam and Nitenpyram are popular. Their usage began from the beginning of 1990 and is increasing till 2008 in Japan. Recent annual total usage of seven insecticides is not increasing, approximately 400 tons per year in Japan. However very little is known about their occurrence, their behaviors and their ecological risk in Japan. Although their monitoring in surface water including storm water, wastewater and sewage treated water, which can be important to Japanese aquatic environment, is necessary to their ecological risk assessment, two big problems must be solved. One problem is their difficult analytical method by using LC/MS/MS. The other is their monitoring method which is constituted by only grab sampling. Grab sampling is a very basic and effective sampling method when the “sampling frequency” is high. However, high frequency is difficult in most investigations and screenings. In this study, a novel monitoring method to measure neonicotinoid insecticides in surface water is developed. This method is constituted of two techniques, passive sampling technique and ELISA assay detection technique. Passive sampling technique is very useful to on-site concentration, accumulation monitoring during deployment and evaluating time-weighted average concentration. ELISA assay detection is very simple, rapid, selective and sensitive determination technique. In this hybrid method, Chemcatcher® and commercial kit are used. This presentation will show comparison between concentration data by LC/MS/MS and those by ELISA technique in grab water samples, calibration tests for sampling rates of neonicotinoid insecticides and their occurrence in surface water in Japan.

WE047

Does the settling of the resin beads have an impact on DGT measurements?

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DGT (*Diffusion Gradients in Thin Films*) is one of the most popular passive samplers for the determination of inorganic and organic fluxes (availability). Recent publications have shown that in presence of partially labile complexes, most of the metal accumulation in the DGT device comes from dissociation of the complex in the resin disc [1]. The distribution of the resin beads (in the resin disc) might, then, play an important role in metal accumulation. For simplicity, it is usually assumed that there is a homogeneous distribution of the binding sites in the resin [2]. However, it is well known that, during the preparation of the resin, the binding beads settle to one side of the disc. The influence of this inhomogeneity on metal accumulation (in presence of ligands) is here assessed using numerical simulation of DGT devices with resin beads in only one half of the resin disc. Results indicate that, due to the settling, there is a deficit in mass accumulation which increases as K (stability constant of the metal complex) increases[3]. The maximum deficit takes place for complexes with lability degrees close to 0.25, while very labile or inert complexes remain unaffected. A maximum deficit of 13% can be found when $Kc_L < 10^2$, but this deficit can increase up to 30% when $Kc_L < 10^3$ or up to 36.7% when $Kc_L < 10^4$ (c_L being the ligand concentration). Additionally, DGT devices with a stack of two resin discs can be used to estimate kinetic dissociation constants of complexes[2;4]. The influence of the inhomogeneity on the recovered kinetic constant is also analysed. It has been found that the recovered kinetic dissociation constant, $k_{d,recovered}$, and the true value, k_d, are practically proportional: $k_d \approx k_{d,recovered}/f$ being f the fraction of volume of the resin disc occupied by resin beads. This relationship is quite independent of the value of K and k_d. Funding from the Spanish Ministry MINECO (projects CTM2012-39183, CTM2013-48967) and from FEDER is acknowledged.
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WE048

Investigation of trace element quantification alteration by fouling development on DGT passive samplers

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The Diffusive Gradient in Thin Film technique (DGT) has been mainly developed for passive sampling of trace elements and its use for water quality monitoring is currently considered. Among the potential biases induced by sampler deployment in water systems, fouling of the protective membrane has been identified as potentially interfering elements diffusion in the sampler. Although numerous *in situ* deployments have been performed in water systems such as rivers, influence of fouling development on element quantification has been nearly not studied. This work proposes an investigation on fouling development on sampler and its influence on element quantification. For this purpose, three contrasted systems are studied: a conventional river, a wastewater impacted river and a stagnant pond. In each system, three types of membrane (cellulose acetate, polycarbonate and polyethersulfone) are deployed several weeks for fooling deposition and development. Membranes are then brought back to laboratory, incorporated into DGT systems and deployed in controlled solution (pH 7 and 10^{-2} M ionic strength fixed with $NaNO_3$) containing metals (Cd, Cu, Ni, Cr(III), Cr(VI) or Pb), metalloid (As) or nutrient (PO_4^{3-}) in order to determine their effective diffusion coefficient in the sampler. In parallel, fouling of the membrane is characterized through elemental analysis and observation with scanning electron microscopy coupled with X-ray microanalysis. Diffusion coefficient derived for fouled samplers will be presented and sampling biases induced by fouling will be quantified. Furthermore, link between diffusion coefficient alteration and composition of the fouling will be discussed.

WE049

Bioavailability assessment of copper in sediments using chemical methods and bioassay and the effects of potential adsorbents in remediation

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Metal contamination in aquatic environments is a growing concern. Bioavailability is the main factor determining toxicity of metals and therefore the use of bioavailability analysis is encouraged. Using adsorbents to reduce bioavailability and transport has been introduced as a novel remediation method for sediments contaminated by metals. In this study, we assessed the use of passive samplers (DGT) and pore water extracts (Rhizon) in assessing the bioavailability of Cu in a natural fresh water sediment. We also compared the results to a bioassay and evaluated the remediation potential and possible adverse effects of two adsorbent materials: chitosan and a biochar. The natural uncontaminated sediment was spiked with $CuCl_2$ (0–460 mg/kg dw) and subsamples were spiked with 0; 0.5 and 5 % (dw) chitosan or biochar. Total Cu concentration and bioavailable concentrations (DGT and Rhizon) were analyzed and the effects of Cu and/or amendments to biota were evaluated with a biotest (*Lumbriculus variegatus*). The functionality of the adsorbents was analyzed using DGT and Cu body residue in L. variegatus. There was a strong correlation between DGT and sediment total Cu concentrations between DGT and Rhizon samples. The growth of *L. variegatus* was reduced in chitosan-spiked sediments, but no adverse effects of biochar were seen. The bioaccumulation or Cu into *L. variegatus* increased together with total sediment concentration. On the basis of chemical methods, 5% chitosan reduced the availability of copper by 75–87 %. For biochar, no remediation potential was seen. DGT and pore water sampling gave comparable results for the bioavailability of Cu and they seem to be usable methods. However, neither chitosan nor biochar reduced the uptake of copper in the test organisms. Most of the studies of adsorbent materials for metals are focused on the chemical analysis of remediation potential, but this study shows that biological responses needs to be taken into consideration as well. The main uptake route of Cu *to L. variegatus* is seen to be through ingestion of sediment particles, which could partly explain the results. Since the uptake route of metals varies between different metals and species, further studies are still needed.

WE050

Does bioavailability of Zn from hydroponic media correlate with the free metal concentration or with the flux of a labile fraction?

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Zinc is an essential element for plant nutrition. In solutions, zinc exists in different chemical forms such as free hydrated ion, inorganic and organic complexes. The bioavailability of elements in the soil solution depends on their chemical speciation. Lipophilicity, global charge and lability of the complexes are the main factors that determine metal uptake by organisms and therefore information about speciation is crucial. For that, it is necessary to develop analytical techniques able to measure not the total metal in solution, but the free fraction or the labile ones. Four techniques, AGNES (Absence of Gradients and Nernstian Equilibrium Stripping)[1], ASV (Anodic Stripping Voltammetry), DGT (Diffusive Gradients in Thin films)[2] and Polymer Inclusion Membranes (PIMs)[3], are combined for this purpose in this work. DGT and PIMs are passive samplers. We present, for the first time, a comprehensive study of the application of these techniques in Hoagland hydroponic medium. AGNES provides the free Zn concentration. DGT, ASV provide availability fluxes associated to different labile fractions. An innovative approach based on PIMs has also been applied after optimization of parameters[4]. Zinc accumulation in the roots and shoots of *Solanum tuberosum* (potato plant) grown in hydroponic media with added Zn, and with or without the addition of organic ligands (humic acid, EDTA) has been measured. Correlations between the amount of Zn accumulated in the plant parts and the free and labile fractions measured in the hydroponic media with the different techniques have been evaluated. Funding from the Spanish Ministry MINECO (projects CTM2012-39183 and CTM2013-48967) is acknowledged. References: (1) Chito, D. et.al. *Sci.Total Envir.* **2012,** *421-422*, 238. (2) Galceran, J. et.al. *Environ.Chem.* **2015,** *12*, 112. (3) Vazquez, M. I. et.al. *J.Membrane Sci.* **2014,** *455*, 312. (4) Almeida, M. I. G. S. et.al. *Environ.Pollut* **2014,** *193*, 233.

WE051

Identification of contamination hotspots along a large river by silicone-based passive sampling

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Silicone-based passive sampling allows the quantification of time-weighted averaged concentrations (C_{TWA}) of hydrophobic organic chemicals (HOCs) in water, even in the low pg L^{-1} range. In previous studies, the suitability of passive sampling for the monitoring of HOCs was tested in different rivers in Germany. Thereby, the sampling site in the river Saar showed higher contamination with polycyclic aromatic hydrocarbons (PAHs) compared to the sampling sites in the rivers Elbe, Saale, Rhine and Danube. The river Saar originates in France and passes the Saarland conurbation before it flows into the river Moselle in Germany. The Saarland conurbation around the city of Saarbrücken is known as a former highly industrialised region with mining, iron and steel industries. In this study, we applied passive samplers for the identification of contamination hotspots along the German part of the river Saar. Silicone rubber sheets were exposed five weeks *in situ* at twelve sampling sites in May/June 2015. Sampling sites were located in the river Saar and its major tributaries, in the effluent of a waste water treatment plant and in the river Moselle after the confluence of the river Saar. Analyte-specific sampling rates (R_s) were determinedby *in situ* dissipation of performance reference compounds (PRCs). In the laboratory, passive samplers were extracted with n-heptane in a Soxhlet apparatus for 24-30 hours and extracts were analysed for polychlorinated biphenyls, dichlortriphenyltrichlorethane and its metabolites as well as PAHs by GC-MS/MS. For the determination of C_{TWA} in water, R_s of target analytes were modelled by non-linear least square regression of the fraction of retained PRCs according to Rusina et al. [1]. Preliminary results show that C_{TWA} of HOCs were mostly in the pg L^{-1} range with single PAHs up to few ng L^{-1}. In general, C_{TWA} of HOCs were higher in the river Saar than in the river Moselle after confluence of both rivers**.** Passive sampling data will be compared to aqueous concentrations determined by conventional chemical analysis of composite samples. Furthermore, the chances and limitations of the application of silicone-based passive sampling for regulatory chemical monitoring in regard to the European Water Framework Directive will be discussed. References [1] Rusina et al. 2010. Calibration of silicone rubber passive samplers: Experimental and modeled relations between sampling rate and compound properties. ES&T 44, 362-367.

WE052

An innovative receiving phase for passive sampling of polar and apolar pesticides in surface waters

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Silicone rubber (SR) has been successfully implemented as a receiving phase in various passive samplers (MESCO, Passive SBSE and silicone rod/sheet). Indeed, SR is mechanically resistant, insoluble in common solvents used for compound desorption, and enable a thermal desorption at high temperature. Nevertheless, SR is an hydrophobic material which permit mainly enrichment of apolar organic compounds (log $K_{ow} > 3$) from water (organochlorine pesticides, PAHs, PCBs, etc.). To remedy this lack of measurement representativity, the use of an other passive sampler in co-deployement for polar organics, as Polar Organic Chemical Integrative Sampler (POCIS), is the stategy preferred. This work aimed at testing the affinity of an innovative material for more polar organic compounds using passive samplers with a single receiving phase. In this way, a **Polar/Apolar Composite Silicone Rubber (PACSiR)** was developed for sorption of a wide range of organics compounds. First, this innovative composite material was assessed in laboratory for pesticides sorption whith a large range of physico-chemical properties. Then, PACSiR was shaped and calibrated as a passive sampler in laboratory, to be finally applied for the monitoring of several pesticides in surface waters. Extraction recoveries determined experimentally for 22 pesticides were similar or higher for all pesticides than for SR. The enhancement of extraction performances is the highest for polar pesticides, with log K_{ow} below 3, which are poorly sorbed on SR. Kinetics of uptake of pesticides in calibration system were mainly described by a pseudo-linear equation model for 14 days exposure. Sampling rate and partition coeficient were determined for calculation of time weighted average concentration (TWAC). Sampling rates for PACSiR passive sampler were 1.3 to 12.5 higher than for SR depending on the pesticide. Consequently, PACSiR enable to reach lower LOQ than SR for polar pesticides. Finally, results of field exposition of passive samplers in rivers were compared with grab sampling to access the occurrence of pesticides and to estimate the TWACs during field exposition. This study demonstrated that the PACSiR is a sensitive receiving phase for pesticides which can greatly improved sorption properties comparared to SR and permitted to reach lower LOQ for polar pesticides. Further experimental investigations on accumulation of other organic compounds will be carried out (hormones, pharmaceuticals, PAHs and PCBs).

WE053

Passive sampling of anionic herbicides (bentazon, chlorsulfuron, ioxynil and mecoprop) by DGT technique

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POCIS (Polar Organic Chemical Integrative Sampler) is the most commonly used passive sampler for the study of polar pesticides (POCIS-HLB) and, recently, of ionic pesticides (POCIS-MAX). Some limitations, regarding the influence of environmental parameters (such as water flow rate) on sampling rates, are however recognized. The DGT (Diffusive Gradients in Thin films) device is less affected by this limitation since uptake from the exposure medium is controlled by diffusion through a diffusive layer. An abundant litterature documents the application of DGT to inorganic compounds such as trace metals or phosphorus and recent developments have been done for few organic compounds, *i.e.* bisphenols, antibiotics, 4-chlorophenol, glyphosate and aminomethyl phosphonic acid. This work aimed at investigating the applicability of a DGT device for the monitoring of herbicides (bentazon, chlorsulfuron, ioxynil and mecoprop) present in an anionic form at natural water pH. DGT diffusive and binding gels were prepared with crosslinked polyacrylamide and OASIS® MAX was used as the binding resin. Pesticide analysis was performed by High Performance Liquid Chromatography combined with Time of Flight Mass Spectrometry. After exposure and dismantling, the binding gel was eluted in a methanol – 1M formic acid mixture (90/10) and the eluted mass was used to calculate the time weighted average concentration (C_{DGT}). Water samples were also collected during the experiments to determine pesticide concentration in bulk solution ($C_{solution}$). Diffusion coefficients of $4.32.10^{-6}$, $3.53.10^{-6}$, $4.97.10^{-6}$ and $4.20.10^{-6}$ $cm^2\ s^{-1}$ were determined at 25°C for bentazon, chlorsulfuron, ioxynil and mecoprop, respectively. The mass of pesticide accumulated by DGT increased linearly with exposure duration up to 10 μg per disk. Bentazon, chlorsulfuron, ioxynil and mecoprop (with pKa values of 3.28, 3.4, 4.1 and 3.11, respectively) are affected by solution pH. For bentazon and mecoprop, the performance of DGT seems to be unaffected by pH in the range 4 to 8. For ioxynil, an increase of pH from 5.0 to 8.0 led to the decrease of C_{DGT} / $C_{solution}$ by appromately 17%. Ionic strength increase was found to slighly affect bentazon and chlorsulfuron sampling only. The tested binding phase was found to be suitable with long term deployments and robust over the pH range of natural waters. DGT appears therefore to be a promising tool for measuring time-weighted average concentrations of anionic pesticides in waters.

Persistent and mobile contaminants in the aquatic environment: how to identify, analyse and regulate a potential threat for drinking water resources (P)

WE055

Using REACH registration data for the identification of persistent, mobile and toxic (PMT) substances to protect raw water resources

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Chemical substances and their uses that fall within the scope of the REACH Regulation (EC No. 1907/2006) have to be registered at the European Chemicals Agency (ECHA) in Helsinki. Registrants are requested to ensure a high level of protection of human health and the environment. By doing so, industry guarantees the safe use of chemicals throughout the whole life cycle. Raw water resources used for the production of drinking water need a high level of protection. In Europe drinking water is obtained mainly from groundwater, reservoirs or river bank filtration. If these environmental compartments are exposed to hazardous chemicals a contamination of drinking water is possible. In the last decade the fate and behaviour of substances in the aquatic environment has been investigated both scientifically and from a regulatory perspective. The finding is that the intrinsic hazard potential of a substance with respect to raw water contamination is maximised if the substance is at the same time persistent in the environment and mobile in the water cycle. Once emitted, these substances remain in the aquatic environment and the contamination is irreparable. If in addition they also fulfil the properties of being toxic any emission into water resources should be avoided during the production and downstream use of these substances. Kalberlah et al. (2014) present a proposal for an assessment concept of persistent, mobile and toxic chemicals (PMT substances). We show that the assessment concept for PMT substances uses the same set of data provided as standard information requirements for the registrations under REACH. It may builds the basis for regulatory measures by authorities. From an authority point of view such a contamination may be of equivalent concern as those substances with persistent, bioaccumulative and toxic (PBT) properties. So far, a potential for raw water contamination by PMT substances is not part of the standardized assessment under REACH. Kalberlah et al. (2014) also published a proposal for a practical guide. Therefor our assessment concept may also be applied by registrants under REACH to fulfil their responsibilities under REACH and ensure a safe use of chemicals.

WE056

Interpretation of Residue Data from a Groundwater Monitoring Study to Define Environmental Safe Use

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*Current regulations governing the registration of plant protection products in the European Union (1107/2009) mandate that an assessment for the potential to reach groundwater is made. A recent Sanco opinion (*Sanco/13144/2010 rev. 3) has *provided guidance on demonstrating "safe use" through monitoring; specifically, 90% of the analyses, obtained from at least 50 locations would need to be less than EU trigger values in order for the Commission to consider a proposal for EU level approval. In 2012, a groundwater monitoring study was initiated with the objective of deriving a statistically valid 90th and 95th percentile concentration for the non-relevant S-metolachlor metabolites CGA354743 (ESA) and CGA51202 (OXA). From 796 quarterly samples collected from 124 locations across 11 European countries over a time period of 12-24 months , for OXA, 40% (321 samples) were < LOQ and >99% (789 samples) were < 10 µg/L. Despite the seven isolated OXA detections >10 µg/L, no site was found to have a long term average >10 µg/L which provides statistically valid confirmation of safe use. For ESA, 16% (126 samples) were < LOQ and 97% were < 10 µg/L (774 samples). Of the samples >10 µg/L (22 samples; 2.7%), only 5 sites were found to have a long term average >10 µg/L with no sites demonstrating analytical residues consistently >10 µg/L. The 90th and 95th percentile of ESA concentration was derived using sampling survey statistics and these were determined to be significantly below the EU trigger. Even using a worst-case assessment of the maximum site average in each relevant FOCUS zone, Châteaudun, Piacenza and Thiva residues were 10 µg/L,this indicated that exceedances are not widespread. The relative vulnerability of the sites was determined by linking the actual field properties back to the original European cumulative distribution of ESA mass flux, placing 35% of the sites above the 90th centile of vulnerability. In conclusion, this exceptionally thorough monitoring study across 124 randomly selected locations supports the "safe use" of S-metolachlor on the basis that both the 90th and 95th confidence intervals for OXA and ESA were < 10 µg/L.*

WE057

Carbamazepine and degradation by products in watershed and effluents

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Carbamazepine, as antiepileptic drug is well-known as anthropic pollutant in WWTP effluent as well as in surface and groundwaters. Together with this ubiquist compound, different studies highlight the occurrence of several degradation products. But origins of the degradation products (degradation in WWTP, in surface water or soil, etc…) are more or less elucidated, due to few works considering all the compartment in the same study. In this work we have considered an alluvial plain impacted by several WWTP, with well-known connections between ground and surface waters. Influent and effluent of main WWTP stations were analyzed monthly to well characterize the pollution sources. Two sampling campaigns have been undertaken for simultaneous pot sampling of groundwater, surface water and effluents. In each case, carbamazepine and its main metabolites (10,11 dihydro 10 hydroxycarbamazepine, 2-hydroxy-carbamazepine, 10,11 epoxide- carbamazepine) and oxcarbazepine were analyzed. In parallel, High Resolution Mass Spectrometric analysis was applied to screen for unexpected degradation by-products. High persistency of carbamazepine and its main degradation products can be highlighted during wastewater treatments, combined with low level of detection and concentration in the watershed. HRMS data give useful information concerning occurrence of unexpected compounds, that allow a better understanding of carbamazepine's fate and behavior.

WE058

Identification of pesticides and their transformation products in groundwater

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Nowadays it is increasingly important for public policy to know what compounds are present in groundwater and which of them must be monitored. This implies the need of specific analytical methodology to identify these micropollutants, emerging substances or transformation products present at low concentrations. The high resolution mass spectrometry (HRMS) has gained increasingly in importance for monitoring these organic compounds. Its high resolving power, mass accuracy and the sensitive full spectrum acquisition are the key points. On the other hand, the main difficulties for the implementation of monitoring are sometimes low and fluctuating concentration levels and complex mixture of pollutants. Therefore there is a strong interest to combine passive sampling to HRMS. Passive samplers allow accumulating compounds during exposure that improve trace detection and integrating pollution fluctuations. The Polar Organic Chemical Integrative Sampler (POCIS) was employed to sampling polar and semi-polar compounds (pesticides, pharmaceuticals, phenolic compounds, triazoles….). Two drinking water supplies were investigated and sampled during several months. Passive sampling was deployed monthly and analyzed by LC-QTof. To process data, different approaches were investigated. The first one is based on research from compounds listed on our homemade database (around 450 with experimental data on our system as retention time, exact masses for molecular and fragment ions) and suspect database (from bibliography and online databases). This suspect list was supplemented by crop protection agents and their degradation products of interest for these sites. The second approach concerns the non-target screening that could give information on the presence of other degradation products or unknown compounds present in all samples. These approaches allow highlighting the use of passive samplers as storage tool because more compounds are identified with POCIS. The use of these two techniques (passive sampler and HRMS) identified some target and suspect compounds present in groundwater on several month.

WE059

Neonicotinoid Pesticides in Drinking Water from Agricultural Regions of the Great Lakes Basin, Ontario, Canada

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Neonicotinoid insecticides (NNIs) are widely used in regions of the Great Lakes basin in Ontario, Canada where there is intensive agriculture. Because of the persistence and high mobility of NNIs, there is concern that these insecticides may contaminate surface waters and groundwater in agricultural regions, including sources of drinking water. The objective of the project was to evaluate the distribution of NNIs and their metabolites in raw (untreated) and finished (treated) drinking waters in selected agricultural regions of southern Ontario, Canada. Raw and finished drinking waters were monitored with Polar Organic Chemical Integrative Samplers (POCIS) and grab samples collected from six drinking water treatment plants that draw their water in agricultural areas. The data from POCIS deployments indicate that there was contamination of raw water at concentrations < 25 ng/L, with clothianidin, imidacloprid, thiacloprid and thiamethoxam being the compounds most widely detected. The frequency of detection of NNIs was much lower in finished drinking water, but some of these compounds were detected with POCIS samplers at estimated concentrations in the low ng/L range. The data from grab samples of raw and finished drinking water collected at the time of POCIS deployment and retrieval, respectively showed good agreement between measured concentrations and the concentrations estimated from POCIS, although concentrations of NNIs in grab samples collected from finished drinking water were all below detection limits. Even though NNIs have been shown to have low toxicity to vertebrates, including humans, contamination of drinking water by this class of insecticides is still a cause for concern.

WE060

Fate of Persistent Organic Pollutants: Surface Water and Sediment

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For monitoring studies the samples of surface water and sediment were taken and analyzed for the probable content of persistent organic pollutants (POPs), organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs) of the Republic of Armenia. The study involved Sevan Lake, rivers Masrik, Martuni, Gavaraget, Hrazdan, as well as water reservoirs "Yerevanyan" and "Akhpara". Gas-Chromatograph/ Mass-Spectrometer GCMS-QP2010 SE EI 230V CELV incl. GC-2010 Plus(Shimadzu Corporation, Japan) was used for analyses. The following pesticides were revealed in studied samples: Hexachlorcyclohexane; DDT; Heptachlor; Hexachlorbenzene; Aldrin; Dieldrin; Endrin; Mirex. As revealed, in all tested samples of surface water the residual amounts of pesticides were revealed at microgram levels. DDT was determined only in Masrik and Gavaraget rivers; in other water basins only products of DDT degradation were found: DDE and DDD. A typical picture of PCBs-related anthropogenic pollution was also observed. The attention should be paid to the presence of great amounts of congeners; this also confirmed the fact of technogenic pollution, viz.: transformer oils were the source of pollution. The peculiarities of POPs transformation along the trophic chains of the hydroecosystem were revealed: the process of PCBs accumulation occurred from the lower to the higher trophic levels. The research performed for determination of POPs content in samples of water, hydrobionts and bottom sediment of theLakeSevanand major rivers ofArmeniaallowed to assess the degree of their bioaccumulation in the hydroecosystem. The high content of POPs in bottom sediment might pose danger for penetration of these substances to human organism – through hydrobionts, detritophages. The process of PCB accumulation most intensively proceeds in hydroecosystems. Being deposited in bottom sediment, through the detritophages, they are involved in the turnover of substances. The research results showed that PCBs content in the studied samples exceeded the hygienic standards. The level of PCBs is dictated by the presence and use of PCB-containing oils in the power-production complex (capacitor, transformer oils, etc.) Monitoring studies revealed presence of PCBs in all tested samples of water from reservoirs of the Republic of Armenia; PCBs content in bottom sediment exceeded the level of these substances in water basins of the country by 1-2 orders of value.

WE061

Biliary PAHs metabolites in red mullet (Mullus barbatus) from Spanish Mediterranean coast

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Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous organic contaminants present in marine environment as a consequence of their continuous input from either land- or marine-based sources. Fishes have a higher capacity to uptake, metabolize and excrete PAHs than invertebrates such as molluscs, therefore the accumulation of PAHs metabolite in their bile fluid can be used to assess the environmental exposure to the parent compounds. In this study the bile concentration of PAHs metabolites in red mullet (*Mullus barbatus*) from 10 areas from the Spanish Mediterranean coast were characterized (Barcelona, Ebro Delta, Tarragona, Mallorca, Valencia, Santa Pola, Cartagena, AlmerCa, Castell de Ferro and Mlaga). Red mullets from 12 to 18 cm were sampled in autumn 2012, 2013 and 2014. Bile samples were treated individually and maintained at -20BC until analysis. In addition the PAHs profile was analyzed in samples of muscle of the same specimens in order to compare the relationships between the PAH bioaccumulation and the PAH bile levels. Phenanthrol and pyrenol were the major PAH metabolites detected in the red mullet bile, and their concentrations were analyzed by liquid chromatography with fluorescence detection using a standard solution for external calibration. Phenanthrol and pyrenol were found in all samples, with pyrenol reaching the highest concentrations. The metabolite concentrations were higher in specimens sampled close to Barcelona than in the rest areas. In contrast, the minimum values were found in Castell de Ferro. These results showed that accumulation of PAHs metabolites was related with the human pressure. Thus phenanthol and pyrenol concentrations in red mullet bile represented better their PAHs exposition in the marine environment than PAHs concentrations in muscle due to the high hepatic metabolism in red mullet.

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WE062

Effects of Clay Minerals on Diethyl Phthalate Degradation

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Phthalate esters are a group of plasticizers used to increase plastic flexibility, transparency, durability, and longevity. They have been detected in China's agricultural and industrial soils. In this study, effects of clay minerals on diethyl phthalate ester (DEP)'s Fenton reaction were examined. The results showed the absorption of DEP to different clay minerals could significantly reduce DEP degradation. The clay minerals with low Fe content would quench free radicals and reduce DEP degradation in solution, and adsorbed DEP would not be degraded. However, the clay minerals with high Fe content would produce more free radical and accelerate DEP degradation in solution, and adsorbed DEP could be degraded. This study implied that clay types, compound structure, exchangeable cation played important roles in phthalate ester's degradation.

WE063

Per- and polyfluoroalkyl (PFASs) contamination profiles in water and sediments for two contrasting lakes in southern and northern Sweden

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Perfluoroalkyl and polyfluoroalkyl substances (PFASs) are becoming an increasingly global concern. PFASs are highly fluorinated organic chemicals that represent a serious risk for environmental and human health. Due to their toxicological impact, PFASs are now included in several emission regulations. Even if the global level of PFASs use is decreasing much of present concentrations in the environment are results of usage and exposure in the past. Therefore, observation of current environmental concentrations and transport patterns may lead to better prediction of their potential environmental distribution, health impact, and future fate. Investigation of PFASs distribution profiles was carried out in surface waters and sediments of the two lakes Sänksjön and Langsjön in southeast and northeast of Sweden. In total 11 sediment cores and 6 water samples were collected to investigate the PFASs concentration by using high-performance liquid chromatograph-tandem mass spectrometer (HPLC-MS/MS). The concentration of PFASs in sediment ranged from 2.7 to 216 ng g^{-1} dry weight for the Sänksjön lake and from 0.8 to 78 ng g^{-1} dry weight for the Langsjön lake. The PFAS concentrations and distribution were correlated to investigate transport conditions for different PFASs and to determine the possible exposure time sequence for each sampling site. Selected water and sediment properties such as pH, conductivity, and total organic carbon were correlated to observed PFAS concentrations. The results for water samples reflect the high contamination of surface water in both lakes. The maximum concentrations were 79 and 1885 ng L^{-1} for Sänksjön and Langsjön, respectively. Analysis of sediment cores reflects the temporal evolution of discharge patterns to the two lakes. The correlation of selected samples suggests transport and degradation mechanisms.

WE064

Evaluation of a suspected-target and non-target high-resolution mass spectrometry method for the preliminary detection of contaminants of emerging concern in water samples impacted by wastewater

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In our continuously growing society, there is a constant production, use, and disposal of products such as antibiotics, flame retardants, personal care products, pharmaceuticals, and pesticides/herbicides. Such products eventually enter the water cycle, where they can persist and negatively affect ecosystems, which represents one of the primary current areas of environmental concern. Further, all of these compounds, which fall under the broad term of contaminants of emerging concern (CECs), can undergo multiple transformation processes, such as photolysis, oxidation, or microbial degradation, and thus be modified into new, and sometimes equally as concerning, compounds. Recent focus has shifted from a targeted analysis, which limits the monitoring to a small set of predefined compounds, to a suspected-target or non-target analysis. This workflow allows for a much more comprehensive screening of analytes, requires no "a priori" knowledge on what compounds are of interest, and allows for the monitoring of transformation products (TPs). The ability of doing such non-targeted analyses, can be mainly attributed to developments in high-resolution mass spectrometry (HRMS), allowing the assignment of molecular formulae, which in combination with other confirming tools (e.g., retention time, isotopic pattern, MS/MS fragmentation data) can be used to identify specific compounds. The current study uses an Orbitrap Q Exactive mass spectrometer, which provides excellent sensitivity and selectivity in full scan, in order to perform suspected-target and non-target screening of CECs and their TPs. The analysis includes

HESI-UPLC-MS full scan analysis at a resolution of 140,000, as well as MS/MS fragmentation data, and the use of the Compound Discoverer software for compound identifications. Two types of sampling methods will be evaluated in combination with this analytical workflow. Firstly, grab samples will be filtered and subjected to a liquid-liquid extraction performed at two pHs (4 and 10) in order to maximize the compound range. Secondly, complementary samples obtained from a polar organic integrative sampler (POCIS) will also be analyzed. This will help to critically assess the capability of this sampling method to obtain polar organic chemicals that may be lost in traditional liquid-liquid extractions.

WE065

Stationary phase selection in HILIC: a study about stationary phase retention properties and the influence of mobile phase parameters

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Persistent and mobile organic micropollutants may pose a threat to drinking and raw waters, and thus need to be monitored and regulated. A high polarity increases the chance of chemicals to be mobile in the water cycle significantly since it leads to good water solubility as well as low volatility and adsorption and thus enables those chemicals to pass most natural and artificial barriers unhindered. The same properties that make organic substances of high polarity potentially relevant for drinking and raw waters also exacerbate their analysis. GC-MS and RP-HPLC-MS, the two predominantly deployed methods in organic trace analysis suffer from insufficient volatility or retention while many alternatives like the use of ion pair reagents, capillary electrophoresis, NP-HPLC and ion chromatography have major drawbacks in combination with mass spectrometry or are limited to ionic compounds. While hydrophilic interaction liquid chromatography (HILIC) has proven to be a versatile tool for the analysis of highly polar organic compounds and is well compatible with mass spectrometry, the technique is not yet widely established. This may be a consequence of the complexity of the multimodal retention mechanism, the availability of a variety of dedicated HILIC stationary phases, the importance of stationary phase selection and the lack of an almost universally applicable stationary phase chemistry such as C18 in RP-HPLC. As a consequence, stationary phase selection is one of the most crucial steps in HILIC method development and in-depth knowledge is required for a suitable selection. Several studies investigated the retention properties of HILIC stationary phases, but most were limited to either analytes of a specific substance class or stationary phases with similar functionality. More broad attempts encompassing a wide range of stationary phases and analyte classes are still rare. In this study we investigated the retention behaviour of 19 model analytes on twelve HILIC stationary phases with different mobile phase compositions. The results allowed a grouping of stationary phases based on their retention properties and selectivity which facilitates stationary phase selection for method development and optimization. A comparison of the retention properties at different mobile phase compositions provides valuable information about the intrinsic optimization potential of important mobile phase parameters, possibly facilitating future HILIC method development.

WE066

Environmental and Metabolic Transformations of the Piscicide, Antimycin A

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Antimycin A is an extremely potent inhibitor of mitochondrial respiration and has been used extensively as a piscicide to eradicate non-native fish species and in aquaculture (e.g., catfish) for the selective removal of scaled fish from ponds prior to restocking. In spite of the long-term use of antimycin A, little information exists on its environmental fate and metabolism. Antimycin A is a bacteria fermentation product containing a nine member dilactone ring and exists as a mixture of eight structural isomers that differ in their dilactone alkyl chain (hexyl or butyl) and acyloxy side chain (butyl or propyl). A high performance liquid chromatographic analytical method was developed for the measurement of antimycin A isomers: $4_{a,b}$, $3_{a,b}$, $2_{a,b}$, and $1_{a,b}$ in aqueous and microsomal samples. Laboratory hydrolysis studies were conducted using certified buffers at eleven different pHs between pH 1 and pH 9. Both static and mixed batch reactors were used, and for the first time first-order abiotic hydrolysis half-lives were measured for individual antimycin isomers in addition to total antimycin. In vitro metabolism studies were conducted using rat hepatic microsomes at pH 7.4. Abiotic hydrolysis of the antimycin lactone occurred more quickly than hydrolysis of the acyloxy moiety. The rate of antimycin A hydrolysis increased with increasing pH. Antimycin $3_{a,b}$ hydrolyzed significantly slower than $1_{a,b}$, above pH 6 while $1_{a,b}$ hydrolyzed more slowly than $3_{a,b}$ below pH 6. At pH 8 and below, the mixed batch systems exhibited faster rates of antimycin A hydrolysis than the static system; above pH 8 the rates were nearly identical for the two systems. In vitro phase 1 metabolism led to the preferential hydrolysis of the antimycin A acyloxy group and deformylation of the N-formylaniline moiety. The four hexyl-containing isomers (1_a, 1_b, 2_a, 2_b) yielded the same metabolite; the four butyl-containing antimycin isomers (3_a, 3_b, 4_a, 4_b) yielded a second unique metabolite. Based on reports that binding of the antimycin A formylamino group is critical for inhibition of mitochondrial respiration (i.e., antimycin efficacy), we hypothesize that the metabolically-mediated deformylation of antimycin A could be a significant pathway for deactivating antimycin A—especially in the case of mammalian exposure (i.e., consumption of antimycin tainted fish). Additional work is ongoing to test this hypothesis.

WE067

Degradation Mechanism of Algal Derived Odorants by Chlorination and UV/Chlorination Processes

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Microbial activities can degrade the quality of drinking water sources by the release of algae derived organic matters including algal toxins and undesirable taste and odor (T&O) causing compounds such as 2-methylisoborneol (2MIB) and geosmin. These T&O compounds have a possibility to be the precursors of trihalomethanes (THMs) as well as other hazardous by-products during chlorination and other treatment processes due to their strong oxidation potentials. Furthermore, the conventional water treatment processes (coagulation, sedimentation, and filtration) are not effective for removing these T&O compounds. For effective treatment of 2MIB and geosmin, the investigation including the identification of their by-products formed during applies processare needed. In this study, chlorination, UV photolysis, and UV/Cl_2 reactions were applied to treat (2MIB and geosmin in water. While chlorination was ineffective to the removal of GSM and 2MIB, UV photolysis and UV/chlorination reactions effectively removed both compounds. This may due to the destruction of tertiary alcohol structure which has a resistant to chlorination. UV photolysis and UV/chlorination reactions followed the pseudo first-order reaction. Neutral pH was effective for the removal of geosmin and 2MIB, due to that OH radical scavenging by OCl^- ion at higher pH. The intermediates identified by GC/MS scan mode (m.w. range 50 to 300) during UV/Cl_2 reaction were 1,4-dimethyl-admantane, 1,3-dimethyl-admantane, and *cis*-1-ethylideneoctahydro-7a-methyl-1H-Indene for geosim, 2-methylenebornane, and 2-methyl-2-bornene for 2MIB). Also, 4-methyl-2-heptanone, 2,4-dimethyl-1-heptene, and 2-methyl-3-pentanol were detected from both 2MIB and geosmin. These intermediates were produced by the rearrangement, bond scission, and ring opening. These intermediates decreased with further reactions, and the formation of chloroform with further reaction. Using the obtained information, the degradation pathways of UV/chlorination of 2MIB and geosmin were constructed.

WE068

Chemical and microbial markers to identify human-associated contamination in Kokemäenjoki watershed

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Microbial and chemical contaminants can be used as source tracking markers in the environment. Accurate identification of pollutant sources in watersheds is crucial for ensuring a good water quality. Ideal human markers are specific i.e. their source is solely anthropogenic. They should also be detectable even at long distances from the source. Sources and distribution of chemical and microbial human markers in Kokemäenjoki watershed, Finland, were studied as a part of Aquatic contaminants - pathways, health risks and management (CONPAT) project. Municipal and industrial wastewaters, surface waters, and artificial ground water were sampled 8-9 times during two years. The feasibility and ability of different markers (i.e. acesulfame, human adenoviruses and human-specific fecal bacteria markers) to indicate human fecal pollution was evaluated. Chemical source markers are typically water soluble compounds that are continuously released to environment at easily detectable concentrations. Artificial sweetener acesulfame is an ideal human marker, since it is commonly used, does not decompose in wastewater treatment, and is persistent in the aquatic environment. It was detected in all studied samples. Fecal indicator bacteria are commonly used for the evaluation of water quality in relation to the potential human health risks. However, the origin of fecal pollution can be either humans or animals and bacterial indicators might decay in the environment with different rate than pathogens such as viruses. Human infecting adenoviruses and human-specific *Bacteroidetes* species are commonly found in sewage and from human-impacted watersheds. We used DNA-based qPCR to characterize human-derived pollution. However, the DNA of the dead microbes may persist in the environment. Metabolically active cells usually contain higher numbers of ribosomes than dormant cells. Therefore we developed RNA-based techniques to characterize active microbial populations. Adenoviruses were common in the CONPAT study area; 62% of the surface water samples contained low numbers of adenovirus gene copies. Human-specific source identifier HF183 was present in all surface water samples. However, the HF183 copy numbers varied greatly depending on the environmental factors such as the diluting effects and distance from the municipal sewage discharge points. Furthermore, the rRNA:rDNA ratio indicating the activity level of the HF183 bacteria group was highest in the most polluted locations.

WE069

Development of a compact continuous submerged water samplers for

monitoring micropollutant time-weighted average concentrations using a 3D printer

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Grab sampling is a very basic and effective sampling method to measure micropollutants in surface water when the "sampling frequency" is high. However, high frequency is difficult in most investigations and screenings. Passive sampling techniques have great advantages to estimation of time-weighted average concentration and accumulation monitoring during their deployment. But passive sampling techniques have also disadvantages to measuring micropollutants in dissolved form and laborious calibration of sampling rates for micropollutants. Recently small continuous low-level aquatic monitoring "C.L.A.M." has been developed which can perform low flow rate extraction sampling at sampling sites. C.L.A.M. is very effective tool to estimate micropollutant concentration in low turbidity surface water. Therefore it is very meaningful to develop novel samplers to monitor micropollutants in particulate and dissolved form even in muddy surface water during several weeks. In this study, a novel sampler which can concentrate dissolved micropollutants at constant flow rates in surface water even with high concentration of suspended solids. Disks which absorb micropollutants in surface water are Empore disks. A filter upon the disk is a 47mm hydrophilic PTFE membrane filter. A flow cell which houses an Empore disk and PTFE membrane is developed using a 3D printer. This presentation will show various developed flow cell models and their filtration characteristics which is very important to build a novel sampler.

WE070

Polycyclic aromatic hydrocarbons in water of Bangladesh

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Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous in nature and widely found in plant, air, soil and water. Anthropogenic activities e.g. motor vehicles, industrial processes, domestic heating, waste incineration etc. are major sources of PAHs to the environment. Due to their carcinogenicity, the water pollution caused by PAHs is a great concern worldwide. Dhaka, the capital of Bangladesh with an inhabitant of 20 million people is vulnerable for the anthropogenic activities. In order to evaluate the level of PAHs, 30 water samples were collected from three big rivers namely Megna, Buriganga and Turag that surround the city and Lake Gulshan within the city. The target polycyclic aromatic hydrocarbons (PAHs) were anthracene, fluoranthene and benzo[a]pyrene. Solid phase extraction using C-18 cartridge was used for the pre-concentration, extraction and clean up, and finally acetonitrile was used for elution of PAHs. High performance liquid chromatography coupled with fluorescence detector (HPLC-FLD; excitation wavelength: 340 nm, emission wavelength: 425 nm) using water-acetonitrile (5:95) isocratic solvent system was used for the identification and quantification of PAHs. The limit of detection (LOD) was 200, 0.63 & 0.63 ng/L and limit of quantification (LOQ) was 660, 2.1 & and 2.1 ng/L for anthracene, fluoranthene and benzo[a]pyrene, respectively. The calibration curves were linear and the correlation coefficients (r^2) were 0.994, 0.997 and 0.998 for anthracene, fluoranthene and benzo[a]pyrene. Good recoveries were obtained for the target analytes (anthracene, fluoranthene and benzo[a]pyrene) and ranged from 91, 85 and 96%, respectively. Analysis of PAHs by HPLC-FLD method has successfully been established after validation. Out of 46 samples, fluoranthene was found in 32 samples in the range of 1.89-1190 ng/L and anthracene was found in 2 samples (1800 and 2900 ng/L). Benzo[a]pyrene was not found in any sample.

WE071

Determination of several organophosphorus triesters in mollusks by pressurized liquid extraction and liquid chromatography-mass spectrometry

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Phosphoric acid triesters are mainly employed as plastic additives, as flame retardants and as plasticizers, but are also used in many other applications, such as in hydraulic fluids, paints and several industrial processes. Many of them are considered as high production volume chemicals and moreover, phosphoric acid triesters usage is growing due to the ban on the use of most polybrominated diphenyl ethers (PBDEs) as flame retardants. When used as plastic additives, phosphoric acid triesters are not chemically bonded but simply mixed with the bulk material they are intended to protect, and so they are relatively easy to release into the surrounding environment. Thus, they have been detected in a range of environmental compartments, like air, water and sediment, and more recently marine biota, which could contribute a health safety issue. Information on these compounds analysis it however limited to very few samples and the most popular triesters only. The aim of this work was the development of a comprehensive method for the extraction and determination of several classes of organophosphorus triesters (including alkylated, arylated and chloro- and bromo-alkylated) in mollusks samples. Pressurized solvent extraction (PLE) integrating a clean-up process was employed as sample preparation technique and liquid chromatography coupled to mass (LC-MS) for separation and determination. The extraction conditions (solvent, sorbents, temperature, time and number of cycles) were optimized maximizing both extraction and clean-up efficiencies. Blank problems associated with the presence of some of the analytes on the filters and on the sorbents were encountered and reduced by using thorough clean-up protocols. Validation of the method was evaluated by the study of linearity, accuracy, precision in terms of relative standard deviation (RSD) and limits of detection and quantification. Finally, the method was applied to different mollusks samples. *Acknowledgement* - This work is financed by MINECO, in the frame of the collaborative project EMERCONFO (CTM2014-56628-C3-2-R). We also acknowledge the support of *Xunta de Galicia* ("Consolidación" funds) and FEDER. We also thank the INTECMAR (Technological Institute for Monitoring of the Marine Environment in Galicia) for providing several mollusk samples

WE072

SimpleBox 4.0: improving the model while keeping it simple...

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Chemical behavior in the environment is often modeled with multimedia fate models. SimpleBox is one often-used multimedia fate model, firstly developed in 1986. Since then, two updated versions were published. Based on recent scientific developments and experience with SimpleBox 3.0, a new version of SimpleBox was developed and is made public: SimpleBox 4.0. In this new model, eight major changes were implemented: removal of the local scale and vegetation compartments, addition of lake compartments and deep ocean compartments (including the thermohaline circulation), implementation of intermittent rain instead of drizzle and of depth dependent soil concentrations, adjustment of the partitioning behavior for organic acids and bases as well as of the value for enthalpy of vaporization. The effects of the model changes in SimpleBox 4.0 on the predicted steady-state concentrations of chemical substances were explored for different substance groups (neutral organic substances, acids, bases, metals) in a standard emission scenario. In general, the largest differences between the predicted concentrations in the new and the old model are caused by the implementation of layered ocean compartments.Undesirable high model complexity caused by vegetation compartments and a local scale were removed to enlarge the simplicity and user friendliness of the model.

WE073

Sediment fingerprinting in the catchment of the Dobczyce Reservoir (South Poland)

G.M. Zemelka,

Excessive fine sediment loadings delivered to a drinking water reservoir from a variety of sources in the human modified catchment have potentially detrimental impacts on aquatic environment and quality of drinking water. Therefore, we attempted to expand the existing state-of-art of the Dobczyce Reservoir (South Poland) research by including sediment fingerprint approach. The Dobczyce Reservoir is situated near Krakow (around 1,000,000 inhabitants) and supplies over 50% of drinking water to the urbanization. It is located in the agricultural and urban catchment. Due to the use of the reservoir as a drinking water source there are several limitations concerning the discharge of wastewater from the catchment. However, the existing studies indicate the presence of pollutants, particularly heavy metals. From the water quality management point of view it is important to determine the sources of pollution and sediments. Among the numerous approaches, the *sediment fingerprinting* has been recently recognized as a valuable tool. The main assumption of this approach is comparison of properties of suspended matter with samples coming from the catchment. In the case of studied reservoir the list of applied sediment fingerprints included a variety of chemical tracers among heavy metals and organic compounds. This *sediment fingerprints* uses chemical tracers, which include, among others: granulometry, radioisotopes, rare earth elements, heavy metals and organic compounds. However, they are expensive, time-consuming and require access to a specialized laboratory. Therefore, in the case of present research it was decided to stress mainly physico-chemical indicators (grain size distribution) and chemical indicators (N and P), heavy metals (Cr, Pb, Cu, Cd, Zn), and selected organic pollutants (PAH and nitro PAH) indicating the origin of the catchments anthropogenic. Research included sampling from the suspension and sediment in three seasons and two repeats (spring, summer, autumn). Sampling took into account the amount of precipitation and flow. This study extended the knowledge of geochemical pollution of the aquatic environment in the Dobczyce Reservoir and showed the source of suspension contamination *sediment fingerprints*.

WE074

Spatial distribution of legacy and emerging contaminants in sediments from the Western Adriatic Sea (Italy)

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FP7 PERSEUS project (Policy-oriented marine Environmental research in the

Southern European Seas) aims to identify the interacting patterns of natural and human-derived pressures on the Mediterranean and Black Seas, linking them to the Marine Strategy Framework Directive (MSFD) descriptors and indicators. Since marine sediments can affect the resident biota, aquatic-dependent wildlife, and human health, the analysis of contaminants in sediments is a key factor for comprehending the overall ecosystem quality. Moreover, the presence of numerous groups of contaminants in sediments are still poorly studied and represent a complex issue in terms of understanding the interaction mechanisms between different chemical compounds and possible environmental consequences. In this context, a monitoring survey (ADREX 14 Cruise) has been conducted in order to investigate spatial patterns of contaminants in sediments from the Adriatic Sea, Italy. Three groups of regulated compounds were analysed: PCBs, DDT (p,p'DDD, p,p'DDE and p,p'DDT) and PAHs. Total PCBs, DDTs and PAHs varied between 0.05 and 4.2 ng g^{-1}, 0.05 and 4.3 ng g^{-1} and 38.8 and 570 ng g^{-1}, respectively. Spatial trens revealed a common pattern, with decreasing concentrations from the northern to the southern Adriatic Sea, suggesting influence from the Po River, which is the major Italian watercourse. Diverse groups of CEC were analysed, such as personal care products (PCPs), which includes fragrances, UV-filters and antimicrobials, organophosphorus compounds and endocrine disruptors compounds. Concentrations of PCPs ranged from-1. Nonylphenol isomers ranged from-1 and organophosphorus ranged from-1. Although CEC also presented higher concentration in the Northern Adriatic, the spatial distribution was not as clear as the one detected for the regulated compounds. Fragrances and UV filters presented some increased concentrations close to turistic areas as the Gargano Promontory, Ancona and Bari, besides the Po River Prodelta area. This work provides an extensive data set on the contamination status of several groups of contaminants in sediments from western Adriatic Sea. Spatial trends suggested the Po River outflow to be a major contributor of pollutants to sediments in the Adriatic Sea. However, the patterns of some PCPs indicate point sources of these compounds, particularly in areas with higher human presence.

WE075

Polycyclic Aromatic Hydrocarbons in the Danube River Sediments: Potential source contributions and carcinogenic risk assessment

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The present investigation was undertaken to investigate potential pollution emission sources and carcinogenic risk of 16 priority PAHs in bottom sediments from the Danube River, Serbia. Three models (Diagnostic Ratios, Cluster Analysis and Principal Component Analysis–Multiple Linear Regression (PCA–MLR)) were applied to analyze the results obtained from ten collected bottom sediment samples. The total concentration of 16 PAHs ranged from 99.48 to 520.48 ng/g dw, with a mean (median) concentration of 271.86 ng/g dw (235.37 ng/g dw). Individual PAH analysis showed that four to six rings PAHs were the most frequently detected isomers and accounted 85% of the total PAHs concentrations. Source apportionment results derived from three different models, considering the whole dataset, were similar, indicating that the highest contribution to ∑PAHs was from: (i) coal combustion, (ii) vehicular emission and (iii) grass and wood combustion. PCA suggested that these three principal components could be the primary PAHs contributors, accounting for 73.7%, 16.0% and 5.5% of PAH concentrations, respectively. Diagnostic ratios showed that PAHs in the sediments of the Danube River were predominantly of pyrolytic origin, followed by petroleum origin (only two sites: ∑LMW/∑HMW=1.57 and 4.66, respectively). According to hierarchical cluster analysis, collected sediments cluster in three major groups, Low (4 localities), Moderate (4 localities) and High (2 localities) contamination that is a consequence of the proximity of the main industries complexes consisting of a petrochemical factory, oil refinery and a chemical fertilizer factory. PAH levels in sediments were compared with Sediments Quality Guidelines (ERL/TEL and ERM/PEL), which suggested that sediment of the Danube River did not show any ecotoxicologial risk for benthic organisms. The values of carcinogenic PAHs were in the range of 26.54 to 277.54 of ∑PAHs. The toxic equivalent concentrations of carcinogenic PAHs were 6.49-74.94 ng/g-BaPeq in Danube sediment, suggesting low carcinogenic risk for this area. **Acknowledgement:** Ministry of Education, Science and Technological Development, Republic of Serbia (III46009) supported this research.

WE076

Identification of pollution hotspots in Norwegian marine sediments using long-term data

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In the present research, we characterized spatiotemporal trends of sediment concentrations of chemical compounds in Norwegian marine waters. To do so, we made use of historical data available through the Norwegian state pollution monitoring programme and the coordinated environmental monitoring programme (CEMP). We used generalized additive models (GAMs) to infer the spatiotemporal trends of the concentrations of PCBs, PAHs and trace metals measured between 1986 and 2008 at 153 sampling locations. GAMs with time, organic carbon content, latitude and longitude as covariates explained ca. 80% of the variability in the log transformed sediment concentrations. For each of the compounds we identified historical pollution hotspots (e.g. Sørfjord (Western Norway) for mercury and zinc and Grenlandfjord (South-Eastern Norway) for PAHs). To identify the local and regional drivers of the contaminant concentrations at these locations we pair the contaminant data with other relevant environmental data (e.g. water chemistry, input data, climatic data and remediation measures). The present research demonstrates that long-term data can provide useful insights in the fate and distribution of chemicals in the environment. The extensive monitoring campaign results in a long series of data, however for some chemicals the design does not allow coherent temporal trend analyses.

WE077

Spatial patterns of target metals (Cu, Pb, Zn) in recent sediments from the Adriatic Sea

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The Adriatic Sea has been facing numerous environmental and climatic challenges due to increasing anthropic pressures, contributing to the input of nutrients and contaminants to this marine system. The final sink for most contaminants is the marine sediment, which can represent the final sink of a complex cocktail of priority substances. Hence, approaches combining multiple contaminants (e.g. metals and polychlorinated biphenyls - PCBs) are extremely important and can contribute to the overall environmental quality status of marine ecosystems. This work was developed under the PERSEUS EU Project (Policy-oriented marine Environmental research in the Southern European Seas), guided by the Marine Strategy Framework Directive (MSFD), which aims to achieve a Good Environmental Status until 2020 in European water bodies. Our aim was to evaluate spatial trends of trace metals (Cu, Pb and Zn) associated to the levels of PCBs in surface sediments of the Adriatic Sea. In order to assess contaminant levels and distribution, a monitoring survey was conducted from northern to the southern Adriatic in October 2014 (ADREX 14). Surface sediments were analyzed for sediment characteristics (e.g. grain size and organic carbon) and target trace metals. Generally, sediment samples collected around the Po River Prodelta (northern Adriatic) presented higher concentrations of metals and PCBs in comparison to the concentrations detected in central and southern Adriatic. This preferential accumulation in the northern Adriatic has already been documented in previous works on the Adriatic Sea and is mainly related to the influence of the Po River discharge, which is the largest Italian drainage basin. Zn and PCBs were positively correlated ($r= 0.60$; $p< 0.01$) between each other, and with organic carbon ($r\geq 0.70$; $p< 0.01$). These correlations suggest a similar distribution of these compounds in the Adriatic Sea, probably driven by the same transport mechanisms and indicating terrigenous input. Trace metals concentrations were above the background levels for the Adriatic Sea (Zn=85; Pb=18;Cu=35 mg kg^{-1}). These elements, mainly Pb and Zn, have a large anthropogenic component in this marine basin, coming from atmospheric inputs and from industrial tailings and wastes. Finally, due to Adriatic Sea semi-enclosed character, metals and PCBs accumulation in the northern part could represent the influence of human activities, which could, in turn, alter the environmental quality status of this European sea.

WE078

Do persistent and mobile organic contaminants fulfil the definition of substances of very high concern owing to their equivalent level of concern defined by REACH

S. Hale, NGI; H. Arp, NGI / Environmental Engineering; L. Vierke, Federal Environment Agency UBA / Section IV Chemicals; M. Neumann, Federal Environment Agency (UBA) / Section IV Chemicals

In Article 57 of the REACH Regulation (1), criteria are given in order to identify substances of very high concern (SVHCs). Within this definition SVHCs can be carcinogens, mutagens and/or reproductive toxicants (CMRs as defined in article 57 a-c) as well as persistent, bioaccumulative and toxic (PBT/vPvB, as defined in article 57 d-e) substances. Furthermore, Article 57 provides a definition of compounds that should be identified as SVHC based upon their equivalent level of concern to CMR or PBT substances and having probable serious effects to human health or the environment (article 57f). The definition of "equivalent level of concern" is open to interpretation. This work investigates whether persistent and mobile organic contaminants (PMOCs) fulfil the requirements and thus can be considered as compounds possessing an equivalent level of concern. In order to investigate whether the requirements are fulfilled, we will focus on which properties of PMOCs could lead to a probable serious effects to human health or the environment at an equivalent level of concern to CMR or PBT substances. This in part is based on the ability of some PMOCs to reach water treatment plants, to resist treatment processes and thus to reach drinking water supplies enabling exposure to humans and thus possible adverse effects on human health. A literature review will identify PMOCs that have reached drinking water supplies based on normal use and not through accidental spillage. One prominent example is that of perfluorinated compounds used historically in aqueous film forming foams to put out fires and which has resulted in contamination of drinking water sources in Sweden and has impacted water supplies in Norway. These case studies will provide a line of evidence to define scientifically why PMOCs are hazardous and will then be used in the context of Article 57f to assess whether PMOCs are of equivalent level of concern and have probable serious effects on human health or the environment. (1) ARTICLE 57: Substances to be included in Annex XIV, TITLE-VII: AUTHORISATION CHAPTER-1: Authorisation requirement

WE079
Fast and accurate screening of small polar organics in the water cycle with UHPLC-QTOF
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The contamination of water sources with anthropogenic polar organic micropollutants (MPs) raises ecotoxicological and toxicological concern. Polar substances can be highly water soluble, highly mobile and can accumulate in aquatic ecosystems when they are not completely removed by natural barriers (e.g. riverbank filtration) and conventional water treatment. Their chemical identities and concentrations observed in the water cycle are constantly changing as a result of societal and climate change, hence reliable tools for the screening of emerging contaminants are needed. The characterization of organic pollutants is essential to assess the potential ecotoxic and toxic effects of individual chemicals and mixtures. In the present work, we developed an analytical method for the screening of small polar MPs with UHPLC-ESI-QTOF-MS. The method was applied to a selection of chemicals having low molecular weights (pH7< 4.5). The selection of the analytes was based on literature data and consisted of 25 polar MPs that have been observed in groundwater, drinking water, and that are not fully removed by reverse osmosis. Liquid chromatography was performed with a core-shell biphenyl stationary phase featuring specific polar retention mechanisms and 100% aqueous stability. Various mobile phase solvents and modifiers were tested, and optimal peak shape and sensitivity were achieved with a fast 7-min linear gradient of 0.05% acetic acid (A) and methanol (B). Water samples were extracted, purified and concentrated using optimized solid phase extraction methodology with hydrophilic lipophilic balanced (HLB) sorbent. The robustness of combining SPE with the UHPLC-ESI-QTOF-MS method was tested with groundwater, drinking water and demi water samples spiked with 50 ng/L analytes prior to extraction. Satisfactory recoveries resulted in LOQs within the sub-ng/L to ng/L range for both matrices. Identification with a high certainty was supported by HR mass spectra. The method was incorporated in a novel software tool for automated target and suspect screening, which allowed fast identification and quantification based on HRMS data. Combining our analytical method with automated screening proved to be an efficient approach to analyze trace levels of a broad range of polar MPs, and can contribute to the characterization of toxic and ecotoxic profiles in the water cycle.

WE080
Diffusion of a dechlorinated metabolite of terbuthylazine in Northern Italian groundwaters
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During a screening campaign on groundwaters used as a source of drinking waters in the area North of Milano, an anxiolytic drug, called Mebikar, has been found in some wells. The origin of this molecules has not been yet identified, but during the tests carried out by high resolution mass spectrometry (HRMS) for confirmation purpose an isobaric compound (MW 198.222360 g/mol and molecular formula $C_8H_{14}N_4O_2$)has been determined in many groundwater samples. The interpretation of the mass spectrum allowed to identify the compound as 4-(*tert*-butylamino)-6-hydroxy-1-methyl-1,3,5-triazin-2(1*H*)-one, which is a described dechlorinated metabolite of the erbicide terbuthylazine, also known as LM6. Other structurally related metabolites were tentatively identified by retrospective analysis via HRMS. These metabolites have been described in the registration document from Syngenta and in the ECHA dossier, but they are neither described in the scientific literature neither registered in CAS. Because ECHA underlined the need for reliable analytical methods for these polar terbuthylazine metabolites, we developed an HPLC-HRMS method based on an Orbitrap system which allowed to determine these compounds in groundwaters and treated drinking waters. In order to characterise the compounds and their diffusion in Northern Italian groundwater we carried out a wide monitoring campaign which highlighted the large diffusion of these compounds. The diffusion has been related to specific soil uses, with special regards to maize cultivation. The results of the occurrence study with literature toxicological data allowed to draft a preliminary risk assessment for these metabolites which should contribute to the derivation of threshold limits for groundwater.

WE081
Screening of chemical pesticides in Swedish surface water and groundwater
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In early 2015 the Swedish EPA was commisioned by the Government to carry out a screening for environmental pollutants including chemical pesticides. A broad screening of chemical pesticides in surface water and groundwater in areas with high agricultural intensity in southern Sweden was therefore requested. This screening program was carried out by the Centre for Chemical Pesticides at the Swedish University of Agricultural Sciences during 2015. The screening was aimed at achieving a better surface coverage compared to the regular national monitoring program and consisted of extensive sampling of both surface water and groundwater. Surface water samples were collected from 46 different sites during May-October, half of the sites were sampled on 5 occasions and half of them on 1-3 occasions. The groundwater sampling was focused on private wells as previous monitoring has indicated that these are more sensitive to pesticide contamination than municipal groundwater wells. The sampling was done in 54 private wells and 18 municipal water works (incoming ground water) on one occasion. Surface water and groundwater samples were analyzed for 131 and 108 different pesticides, respectively. The results include information on which pesticides are the most frequently detected in Swedish surface water and groundwater, as well as on exceedances of the drinking water limit or ecotoxicological guideline values. The results are also analyzed in the light of collected metadata to explore potential correlations with elevated concentrations of pesticides. For surface water correlations with the portion of agricultural land and the size of the drainage area are analyzed. For groundwater relationships to the depth of the well, the distance to agricultural fields and the age of the well are examined. Correlations are also made with well water concentrations of nitrate and *E. coli* as possible signs of well vulnerability. The preliminary results from the screening program are largely consistent with results seen within the regular, spatially limited, national monitoring program. The final results are currently being analyzed and will be reported to the Swedish Government by mid-March 2016. A comprehensive evaluation of pesticide occurrence in Swedish waters will be available for poster presentation.

Antibiotics and Antibiotic Resistance in the Environment: Ecological Fate and Effects, Resistance Development and Implications for Human Health (P)

WE082
Chronic toxicity of cephradine and cefadroxil in Daphnia magna and freshwater fish Oryzias latipes
B. Kim, Department of Environmental Health; K. Ji, Yongin University
Pharmaceuticals in the environment are of growing concern for their potential consequences on human and ecosystem health. Cephradine and cefadroxil, two cephalosporin antibiotics for animals, have been frequently detected in freshwater environment, however their toxicological information is very limited. In the present study, the adverse effects of chronic exposure to cephradine and cefadroxil were evaluated using *Daphnia magna* and *Oryzias latipes*. Chronic toxicity tests with *D. magna* was conducted as outlined in the OECD test guideline 211. *D. magna* were exposed to cephradine and cefadroxil (0, 6.25, 12.5, 25, 50 and 100 mg/L) for 21 d. Fish embryos were exposed to cephradine and cefadroxil (0, 0.001, 0.01, 0.1, 1 and 10 mg/L) for 30 dph. Thyroxine (T4) levels in juvenile fish were measured using enzyme-linked immunosorbent assay (ELISA), and transcriptions of 8 genes in the hypothalamic-pituitary-thyroid (HPT) axis (*trh*, *trhr*, *thrα*, *thrβ*, *tshr*, *tg*, *dio1*, *dio2*) were also measured using real-time polymerase chain reaction (qPCR). The no observed effect concentrations (NOECs) based on the endpoints of survival, reproduction, and growth in *D. magna* exposed to cefadroxil and cephradine were 100 mg/L, except growth NOEC for cephradine (50 mg/L). Survival and body length in juvenile fish were significantly decreased at ≥0.1 mg/L cephradine. While no significant change was observed in T4 hormone, the transcriptions of *tshr* and *tg* genes were significantly altered by exposure to cephradine. These results support that cephradine has the potential to inhibit the normal growth in macroinvertebrate and early-life stage fish, and these were accompanied by an inhibition of mRNA expressions related to thyroid hormone endocrine system. Concentrations of the pharmaceutical studied that have been reported to occur in rivers of Korea are much less than the

thresholds for effects on the endpoints studied here. Further investigations on endocrine disruption potential in aquatic organisms exposed to environmentally relevant concentrations are recommended. \n *Acknowledgement* – This study was supported by National Research Foundation of Korea (Project no. NRF-2013R1A1A1061684).

WE083

Evaluation of potential effect of veterinary pharmaceuticals in concentrated animal feeding operation (CAFO) of Korea

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There is a growing concern about the effects of pharmaceuticals and other pollutants from concentrated animal feeding operation (CAFO), since farm wastes often reach the environment without appropriate treatment. However, limited information is available on monitoring of antibiotic residues and potential risks in water environment around the animal farms. In the present study, potential toxicity on steroidogenesis in water extraction samples were evaluated using H295R cell lines. Measurement of veterinary pharmaceuticals and ecological risk assessment were also performed. Water samples were collected from five locations in a stream that runs alongside a CAFO area in Yongin, Korea. Several upstream and downstream locations were sampled for ambient water during June (low-flow) and August (high-flow), 2013. Total 12 pharmaceuticals (acetaminophen, cephradine, ibuprofen, mefenamic acid, six sulfonamides, and two tetracyclines) were evaluated using HPLC-MS/MS. The effects on the hormone production and expression of mRNAs involved in steroidogenesis were determined with H295R cell bioassays. Hazard quotients were derived from the highest measured environmental concentrations and predicted no effect concentrations. The pharmaceutical concentrations measured near CAFO area were greater than those in upstream or far downstream areas, suggesting that elevated pharmaceutical concentrations in waters are associated with activities of CAFO. Except one sampling area, the level of detection of the target pharmaceuticals were decreased in the high-flow season. However, greater production of 17β-estradiol (E2) was observed in the samples collected during the high-flow season, suggesting that possibly non-point source contaminants other than pharmaceuticals from CAFO may also cause endocrine disruption. Hazard quotients for several pharmaceuticals including acetaminophen exceeded unity, suggesting potential ecological effects in this area. *Acknowledgement* – This study was supported by National Institute of Environmental Research of Korea.

WE084

Effects of temperature, genetic variation and species competition on the sensitivity of algae populations to the antibiotic enrofloxacin

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Primary producers, particularly cyanobacteria, are amongst the most sensitive organisms to antibiotic pollution in aquatic ecosystems. In this study we investigated the effects of the fluoroquinolone antibiotic enrofloxacin on the cyanobacterium *Microcystis aeruginosa* and the green algae species *Scenedesmus obliquus*. The toxicity of the antibiotic was investigated in the laboratory under different temperature conditions (20 and 30°C) and using three different strains of each species. Furthermore, it was investigated how antibiotic pollution affects the competition between *M. aeruginosa* and *S. obliquus*. The competition experiment was performed using three competition treatments, defined as density ratios (i.e. initial bio-volume of 25/75%, 10/90% and 1/99% of *S. obliquus*/*M. aeruginosa*, respectively), one *S. obliquus* control (100% *S. obliquus*) and one *M. aeruginosa* control (100% *M. aeruginosa*), and four different ENR concentrations (i.e., control, 0.01, 0.05 and 0.10 mg/L). In all experiments, growth inhibition based on cell number, bio-volume, chlorophyll-a concentration, as well as PSII efficiency were used as evaluation endpoints. In the majority of the cases PSII efficiency was found to be the most sensitive endpoint, followed by growth inhibition based on cell number. *S. obliquus* was found to be slightly more sensitive at 20°C than at 30°C (EC50-72h of 38 and 41 mg/L, respectively), whereas an opposite trend was observed for *M. aeruginosa* (0.047 and 0.037 mg/L, respectively). Differences in EC50s between strains were within a factor of two. The competition experiment showed that *M. aeruginosa* growth can be significantly reduced at 0.01 mg/L in the presence of *S. obliquus* at a density ratio of 25/75% *S. obliquus*/*M. aeruginosa*. The results of this study confirm the high sensitivity of cyanobacteria to antibiotics and show that temperature and genetic variation can influence their response to them. Furthermore, the results of the competition experiment suggest that the structure of primary producer communities can be affected at antibiotic concentrations close to those that have been monitored in the environment.

WE085

Assessing sensitivity to antibiotics in freshwater cyanobacteria and microalgae

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Cyanobacteria (CB) and microalgae (MA) play fundamental roles in primary production and nutrient-cycling in aquatic systems. Disruption to these communities through chemical exposure can therefore have far-reaching ecosystem consequences. Antibiotics (AB) cause toxicity to prokaryotic cells. Although there are examples where the drug targets appear to be evolutionarily conserved in some eukaryotes, for example in MA that have chloroplasts, the vast majority of the targets are not conserved. The likely consequence is that eukaryotes such as metazoans will not show adverse effects to ABs at exposures of environmental relevance. Environmental risk assessment (ERA) for the registration of AB requires ecotoxicity testing with daphnia and fish but encourages testing for a single species of CB for prokaryotes, as an alternative to the test with a MA. Current ERA of ABs therefore may not be protective for prokaryotes as a whole. Given their importance to ecosystem services we argue that wider understanding on the effects of ABs on prokaryotes and algae is required. It has been suggested that the ERA of ABs should evaluate risk of antibiotic resistance and the predictive no effect concentration may be based on minimum inhibition concentrations (MIC) in clinically relevant bacteria. Increased knowledge regarding differences in species sensitivity will help ensure MIC based ERA will be protective for environmentally relevant bacteria. On the premise that knowledge of variation in species sensitivity to antibiotics in CB and MA is important to better inform current ERA practice for the protection of prokaryotes and algae, we investigated the effects of 3 ABs with different MOAs (ciprofloxacin, sulfamethoxazole and cefotaxime) on 3 CB and on 2 MA. We set out to determine whether one species of CB is representative of a diverse class of taxa and across different MOAs. Inhibition of population growth (a standard regulatory endpoint), expression of relevant target genes (via qPCR) changes in the cell phenotype (through TEM), and bioavailability were for each species and AB studied. Significant differences in the growth rate for the different AB exposures were observed between the CB and MA. Moreover, the three CB species varied in their sensitivity to the AB effects by up to one order of magnitude. Data will be presented illustrating how sensitivity in responses relates to bioavailability and pharmaco-dynamic properties of each antibiotic.

WE087

Selection for Antibiotic Resistance in Complex Microbial Communities

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Antibiotics have been regularly used since the 1900's to treat bacterial infections and have revolutionised modern medicine. However, after development of a particular antibiotic class, resistance genes to counteract the effect of the antibiotic have always emerged. Therefore, there is potential to return to a pre-antibiotic era where treating bacterial infections becomes impossible. Antibiotics are found throughout the environment. They are naturally produced by certain bacterial species; however human activity also leads to the release of measurable quantities into environmental systems. Release into the environment can occur through use of antibiotics in the clinic, as the more stable compounds are excreted into water systems. However, other sources include through use of antibiotics therapeutically and as growth promotors in agriculture, release from pharmaceuticals and use in personal care products. Antibiotics in the environment have the potential to exert a selective pressure on environmental bacteria causing resistance. Recently (2015), antibiotics have been added to the priority substances watch list of the Water Framework Directive for the first time. Macrolides, the group added, have been found in concentrations of up to 90μg/L within water environments. This study will investigate the evolution of macrolide resistance in aquatic systems by studying the genetic mechanisms of macrolide resistance in complex microbial communities and studying experimental evolution of macrolide resistance using culture independent and next generation sequencing methods.

WE088

Minimum inhibitory concentrations and antibiotic resistant genes in the freshwater cyanobacteria Planktothirx agardhii

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Objectives: Cyanobacteria are ubiquitous prokaryotes in aquatic ecosystems and although they can be exposed to antibiotics and antibiotic resistant bacteria, their role on water resistome was never investigated. *Planktothrix agardhii* is one of the

most common cyanobacteria species in Portuguese freshwater reservoirs, often exhibition long residence time in those reservoirs. This work aimed to evaluate the antibiotic susceptibility patterns and resistance mechanisms in *P. agardhii* in order to assess their putative contribution to the global pool of resistance determinants in freshwater. **Methods:** We investigated 8 strains of *P. agardhii*, previously isolated from different freshwater reservoirs. The antibiotic susceptibility was evaluated by a microdilution method previously adapted for cyanobacteria, against beta-lactams, aminoglycosides, quinolones, trimethoprim and tetracycline. Minimum inhibitory concentrations (MIC) were determined according to cell density (DO, 450nm) and microscopic examination of cultures integrity. All strains were searched for antibiotic resistance genes and class 1, 2 and 3 integrons by PCR/sequencing. **Results:** The results showed that *P. agardhii* is not susceptible to trimethoprim and quinolones within the tested concentrations (0.0015-1.6 mg/L). However, the cell growth is strongly inhibited by amoxicillin (median MIC value of 0.1mg/L). The other antibiotics presented the following median MICs: ceftazidime, 1.6 mg/L; ceftriaxone, 0.8 mg/L; tetracycline, 0.4 mg/L; kanamycine, 0.2 mg/L and gentamicine, 0.1mg/L. None of the *P. agardhii* strains exhibited genes associated with trimethoprim and quinolones resistance, which suggests that these cyanobacteria are intrinsically non susceptible to this antibiotics. Conversely, genes associated with streptomycin (*str*A-*str*B) and sulfonamide (*sul*1) resistance, as well as a 1-type integron, were detected in three of the strains. These strains were isolated from the freshwater reservoirs where *Planktothrix* blooms are frequent and. This suggests that the presence of a common antibiotic resistant determinant in *P. agardhii* might be a result from a similar selective pressure within those reservoirs. **Conclusions:** The presence of antibiotic resistance genes and integrons, as well as the reduced susceptibility to antibiotics, suggest that cyanobacteria may play a role on freshwater resistome and eventually contribute to the dissemination of antibiotic resistance in freshwater environments.

WE089

Antimicrobial susceptibility of E. coli isolates from meat industry wastewater

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Wastewater originating from the meat processing industry is characterized by the high presence of organic waste load and potentialy pathogenic microorganisms.*This study consisted of four sampling campaigns, during four seasons in 2013 and 2014.Wastewater was collected from three meat processing plants in the Province of Vojvodina, Republic of Serbia.* The aim of this research was to determine the posible resistance of isolated *E.coli* strains to selected antibiotics.The evaluation of the antimicrobial susceptibility was performed on 37 strains of *E.coli* to 9 different antibiotics.Antibiotics, as emerging pharmaceuticals, used for susceptibility testing were: ampicillin, cefotaxime, ciprofloxacin, chloramphenicol, gentamicin, nalidixic acid, streptomycin, tetracycline, and trimethoprim-sulfamethoxazole.Monitoring of antimicrobial resistance in commensal bacteria *E.coli* is of great significance because this species is commonly present in animal faeces, and can often acquire conjugative plasmids from other present enteric bacteria, thus serving as a valuable reservoir of resistance genes which can be transferred to other bacteria present in same environment. From a total of 37 strains of *E.coli*, a moderate degree of resistance was shown to tetracycline (37.83%); low degree of resistance to ampicillin (21.62%), streptomycin (24.32%), trimethoprim-sulfamethoxazol (18.92%) and nalidixic acid (16.22%); a very low to: chloramphenicol (13.51%), ciprofloxacin (2.7%), gentamicin and cefotaxime (0.0%). Ten of the tested *E.coli* strains (18.92%) were multidrug-resistant. Antibiotics used in this experiment are present on the NORMAN list of emerging substances of concern, since they are most frequently detected in aquatic environment. Due to their detectable presence in the environment, we have to be very careful because it increases the possibility for antimicrobial ressistance in microorganisms which have been in contact with antibiotic residues. High public pressure considering the consequences of mass appearance of multi-resistant bacterial strains that have spread from the animals to humans, managed to significantly reduce the use of antibiotics, especially as growth promoters.Serbia also adopted this EU legislation but more stringent control of law implementation is needed. **Acknowledgement**This work has been done within the NATO project (Ref.984087), and it has also been financially supported by Ministry of Education and Science, Republic of Serbia (Project No.46009).

WE090

Presence of multiple antibiotic resistant Enterococcus spp. harbouring virulence genes in WWTP effluent and receiving water systems in the North West Province, South Africa

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Faecal contaminants from poor operating waste water treatment plants pose a health risk to humans and animals. The latter is heightened when multiple antibiotic resistant bacteria harbouring virulence genes are present. The aim of the current study was to determine the antimicrobial resistance patterns and presence of virulence genes in *Enterococcus* spp. isolated from the final effluent of three municipal wastewater treatment plants and receiving water systems in the North West Province, South Africa. Sixty three *Enterococcus* isolates were isolated and antimicrobial susceptibility test performed on all isolates. Antibiotic inhibition zone diameter data was subject to cluster analysis. The cluster composed of *Enterococcus* spp. from all WWTPs final effluent was predominated by *E. faecalis* spp. followed by *E. faecium* and *E. hirae* spp. Whereas the cluster composed of *Enterococcus spp.* from downstream sites of receiving water systems was predominated by *E. gallinarum, E. casseliflavus* and *E. mundtii*. All 63 *Enterococcus* spp. were screened for the presence of five virulence determinants (*asa1, cylA, esp, gelE* and *hyl*). All five virulence genes were detected and six multi-virulence profiles observed. Analysis of the antimicrobial susceptibility of the 63 *Enterococcus* isolates revealed that resistances to Ampicillin (67%), Vancomycin (62%), Tetracycline (58%), Penicillin (52%) and Erythromycin (51%) were most frequent. Sixty eight percent of the screened *Enterococcus* spp. were resistant to three or more antibiotics. Seventy six of the screened *Enterococcus* isolates resistant to multiple antibiotics had plasmids. Differences in MAR phenotypes were observed for all profiles pre- and post-plasmid curing with lower resistance profiles post plasmid curing. This study has demonstrated that *Enterococcus* spp. harbouring virulence factors and plasmids that mediate multiple antibiotic resistance are present in WWTPs final effluent and receiving water systems that support a variety of social needs in South Africa. Thus, it is recommended that *Enterococcus* spp. be used as an additional faecal indicator in conjunction to *E. coli*. **Keywords:** *Enterococcus* spp.; WWTP final effluent; multiple antibiotic resistance; virulence genes

WE091

Antibiotic-resistant bacteria from air samples collected in nursing homes

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Introduction Antibiotic resistance is currently a worldwide public health problem that can include diverse mechanisms and bacterial species. The understanding and monitoring of such phenomena is essential to avoid further dissemination. The main aim of this study was the characterization of antibiotic resistance mechanisms in bacterial strains collected in air samples from nursing homes and compare them with clinical samples isolated from the same geographic area. **Materials and methods** Air samples were collected from bedrooms, living rooms and outdoor of 4 nursing homes located in Lisbon, Portugal. Screening of antimicrobial susceptibility of 18 Gram-negative (5 *Acinetobacter* spp., 1 *Klebsiella oxytoca*, 4 *Pantoea* spp., 7 *Pseudomonas* spp., 1 *Sphingomonas paucimobilis*) and 12 Gram-positive (3 *Micrococcus luteus*, 3 *Staphylococcus* spp.) isolates was performed by disk diffusion method. Different antibiotic resistant genes were searched by PCR. PFGE was used to evaluate clonality between *K. oxytoca* isolated from the air environment and other strains from clinical origin. **Results and discussion** The majority of strains were susceptible to all antibiotics tested. Among beta-lactam antibiotics, reduced susceptibility to cefoxitin was detected in *Staphylococcus capitis*, through expression of the *mecA* gene, and to ampicillin, piperacillin and piperacillin-tazobactam in 1 *K. oxytoca* expressing a bla_{OXY-5}-type beta-lactamase. Nonsusceptibility to meropenem and piperacillin-tazobactam was observed in 2 *Pseudomonas putida* however no antibiotic resistance gene was detected. Regarding quinolones, non-susceptibility was found in 1 *S S. capitis*, 2 *Staphylococcus haemolyticus*, and 1 *K. oxytoca*. The molecular characterization of the *mecA*-positive *S. capitis* from the nursing homes and from a hospital within the same region suggests a potential dissemination of strains between these two environments. The genetic relatedness of *K. oxytoca* from nursing homes (n=1) and clinical isolates (n=9) recovered within the same region, allowed to conclude that they were not genetically related. **Conclusions** Globally, nursing homes environments may act as complementary reservoirs of antibiotic resistant bacteria and antibiotic resistance genes. Thus, a better understanding of the antibiotic resistance mechanisms and dissemination pathways in other reservoirs than human is essential to control its emergence and spread.

WE092

Antimicrobial susceptibility and integron diversity in organic and conventionally grown fruits and vegetables

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Introduction: During growth and harvesting fresh produce can become contaminated not only with environmental, but also pathogenic bacteria from animal and human sources. This study aimed to characterize the antibiotic resistant bacteria gathered from organically and conventionally grown fruits and vegetables, and to characterize the diversity of class 1, 2 and 3 integrons detected. **Methods:** Between 2013 and 2014, one conventionally and one organically produced batch of six fruits and vegetables were purchased at retail stores. Products were processed as follows: 50g of product was selected at random without washing or peeling, diluted and homogenized. The selection of resistant Gram negative bacteria was performed in VRBG plates containing ten different antibiotics separately. The isolates were identified through the amplification of the 16S rDNA. Antimicrobial susceptibility was assessed by disc diffusion method using EUCAST guidelines. All isolates were investigated for the presence of class 1, 2 and 3 integrons, through PCR amplification, and their variable region was explored by a strategy of Next Generation Sequencing. **Results:** A total of 333 isolates showing nonsusceptibility to, at least, one antibiotic were collected among 144 samples. Among others, *Enterobactericeae* (n=184), *Moraxellaceae* (n=88), *Pseudomonadaceae* (n=36), were detected. Nonsusceptibility was mainly identified among β-lactam antibiotics for *Enterobactericeae* recovered from conventionally produced samples (45.1%); overall, cefoxitin was the most ineffective antibiotic (19.6 % for organic and 26.6% for conventionally grown produce). The molecular screening for the integrase-encoding gene showed the presence of eight isolates with class 1 (2 *Enterobacter*, 2 *Escherichia*, 1 *Morganella*, 2 *Acinetobacter* and 1 *Pseudomonas* spp.), three isolates with class 2 (1 *Escherichia* and 2 *Raoutella* spp.), and one isolate with class 3 integrons (*Klebsiella* spp.). The integrons revealed a variety of gene cassettes conferring nonsusceptibility to different classes of antibiotics. **Conclusion:** Resistance was more frequent in the products grown in close contact with the soil. Besides environmental bacteria, many pathogenic agents were also detected. Lettuce was significantly associated with the existence of class 1 and 2 integrons regardless of the origin. Both organic and conventional produced fruits and vegetables may constitute potential sources of resistant bacteria and of integrons.

WE093

Bioavailability - A concept to unravel the antibiotic effects on soil microbial functioning? Authors & affiliations:

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The intensive uses of antibiotics (AB) in human and veterinary medicines contributed to widespread environmental contaminations, through the wastewater discharges and the recycling of organic wastes (OW) as soil amendments. While the occurrence and persistence of AB in the environment have become major environmental and human health issues, their ecotoxicological impacts are not well understood. AB inhibit active microorganisms and therefore can represent an important risk for the environment, especially for soil microbial functions (organic matter degradation, nutrient recycling). However, these side-effects depend on the bioavailability of AB. Organic matter quality is a key environmental factor influencing soil dissipation processes of AB which can control the bioavailability. Our objectives were to investigate the effects of the sulfamethoxazole (SMX) on microbial nitrogen transformations in relation with AB bioavailability in soil. A dose-effect approach was performed with different doses of SMX supplied to two different OW (compost of sewage sludge / green waste (SGW), farmyard manure (FYM)) before their application to soil. The final doses of SMX in soil microcosms ranged from 0,022 to 2,22 mg kg^{-1}_{dw}, with control soils amended with OW without SMX. Nitrogen forms, potential nitrification and denitrification activities were determined in soils, after 7, 28 and 84 days following amendments. Also, the total amount of SMX and the chemically available fraction were extracted respectively with organic and aqueous solutions and then quantified by UPLC-MS/MS. At these low doses, only nitrification activity was adversely impaired by SMX, following a dose-response pattern, in soils amended by SGW. Actually, as the SMX is a bacteriostatic antibiotic, the effects were stronger for growing microbial populations, such as the nitrifiers which are largely promoted by the high levels of NH_4^+ in SGW amended soils compared to FYM amended soils. The chemical assessment of SMX availability did not show strong differences between the SGW or FYM amended soils. However, throughout the experiment, the decrease of SMX availability was in accordance with a partial recovery of soil nitrification in SGW amended soils. Nitrification is a sensitive indicator of AB effects on soil microbial functioning. Its recovery after AB exposure may be explained by a strong decrease in the chemical availability and consequently in the bioavailable fraction of AB for microorganisms

WE094

Bacterial community structure and biogeochemical activity in soil irrigated with treated wastewater in Tunisia

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In semi-arid regions, agricultural activity relies on water availability. For instance, during the dry season, from June to September in Nabeul region (Tunisia), the irrigation of citrus trees with treated wastewater is an old practice.Conjunctive use of groundwater (salinity 2-5 g/L)with treated wastewater may alsotake place. In this work, we focus on the effect of emerging substances, possibly present in the irrigation water, on soil bacteria diversity and function. Three irrigated plots were selected based on the type of irrigation water: treated wastewater, groundwater, and treated wastewater and groundwater together. Soils were sampled at 0-5 and 5-20 cm depth in June, July, August, and October 2015. Protein production (3-H leucine incorporation) and mineralization of different carbon sources (Biolog) were measured to assess activity of the microbial community in soil samples. Abundance of the universal marker (16S rRNA) and of nitrate-reducing bacteria (*narG* and *napA*) was assessed by qPCR. Presence of the ammonium oxidizing bacteria was assessed by the *amoA* gene detection by PCR. Biodiversity was assessed with a fingerprinting technique (CE-SSCP). Forty emerging substances, including human and veterinary antibiotics, were assessed in groundwater and treated wastewater by HPLC/MS-MS. Preliminary results showed that caffeine, carbamazepine and its metabolite 10,11-epoxycarbamazepine, ketoprofene, ofloxacine, propranolol hydrochloride, and sulfamethoxazole were quantified in both ground and treated waters at concentration higher by 2 orders of magnitude in the treated water (n=4). The results allowed determining whether soil microbial communities are affected by irrigation water and their implication in biogeochemical cycle.

WE095

Removal Characteristics and Mechanism of Antibiotics in the Constructed Wetlands

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The occurrence and removal of antibiotics (sulfamethoxazole (SMZ), sulfathiazole (SFI), sulfamethazine (SMA), trimethoprim (TMP), tetracycline (TC), oxytetracycline (OTC), chlortetracycline (CTC), and enrofloxacin (EFX)) using a constructed wetland (CWs) for treating livestock wastewater were investigated. The levels of antibiotics in the effluents of the CWs were in the order of CTC, SFI, SMZ, SMA, TMP, OTC, EFX and TC, ranging from 47.98 to 6834.66 µg/L, respectively. There was an inverse correlation ($p < 0.0493$) between the removal of sulfonamide group (SMZ, SFI, and SMA) and tetracycline group (TC, OTC, and CTC) antibiotics in the effluents of the CWs, indicating that sulfonamide-type antibiotics were more effectively removed in the CWs. Sulfonamide-type antibiotics have higher pKa values, resulting in more effective adsorption into negatively charged soils through electrostatic interaction. Therefore, the physicochemical properties (e.g., molecular weight, pKa value, ionic bonds, and functional groups) can be the important factors in CWs. Sunlight photo-degradation experiment showed that EFX was effectively removed (70%) compared to other antibiotics. The microcosm adsorption experiments using wetland soils under biotic and abiotic conditions showed that antibiotics in biotic system were more effectively removed than abiotic systems, indicating that soil-mediated microbial degradation is a major removal mechanism. The microcosm adsorption experiments using wetland plants (Phragmites australis) showed that the biotic system also removed sulfonamide-type antibiotics more effectively compared to the abiotic system. Our results suggest that the removals of antibiotics in the CWs are mainly mediated by biodegradation and adsorption onto soil and plants. This study implies that the CW system can be used for the removal of antibiotics for seondary livestock wastewater treatment.

WE096

Direct Photolyses of Ofloxacin and Sulfamethylthiazole

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Due to large scale uses in animal husbandry and aquaculture, ofloxacin (OFL) and sulfamethylthiazole (SMZ) are representative antibiotics found in the surface waters in China, and photolysis is considered as an important pathway for their degradation in nature. In this study, the direct photolysis of OFL and SMZ under simulated sunlight (SL) and mercury vapor lamp (MPL) were investigated with an Agilent Technologies 1260 Infinity HPLC as the analytical tool. Depending on the light intensity of the SL and MPL, typical half-lives of these two synthetic antibiotic compounds were in the range of minutes and the reactions seemed to follow first order kinetics. Mixed aqueous solution of OFL and SMZ was exposed to SL and MPL, respectively, and it was found that SMZ underwent faster photolysis than OFL, but the rates of degradation were slower in comparison with their single component individual rates. The presence of hydrogen peroxide or molecular oxygen in the aqueous solutions seemed to accelerate the photolysis for both the OFL and SMZ. MPL emitted more UV radiation than the SL and tended to expedite the photolysis. The diode array detector of the HPLC system detected the existence of several photolysis products, and ion chromatography analysis of the photoproducts of OFL with a Metrohm 790 IC indicated the presence of fluoride, suggesting that fluorine atom was removed from the molecule of OFL. Dihydoxyl-ofloxacin (i.e. 8-Fluoro-3-hydroxy-9-(4-hydroxy-piperazin-1-yl)-6-oxo-2,3-dihydro-6H-1-oxa-3a-aza-phenalene-5-carboxylic acid), and hydroxyl-ofloxacin (i.e. 8-Fluoro-3-hydroxy-9-(4-methyl-piperazin-1-yl)-6-oxo-2,3-dihydro-6H-1-oxa-3a-aza-phenalene-5-carboxylic acid), defluoro-ofloxacin, demethyl-ofloxacin were suggested as the possible photolysis products and the analogues of the original antibiotic. Computations of the proton and carbon-13 NMR (nuclear magnetic resonance) chemical shifts (termed as NMR indices) of the possible photolysis products of OFL revealed the persistent molecular structure typical of quinolone, and the significance of such feature is discussed in terms of bacteria quinolone-resistance and nucleotide sequences of DNA gyrases in the clinical isolates publicized in scientific journals. It is postulated that sub-lethal exposures to the analogues helped bacteria develop resistance to original compounds. The NMR index of a given compound was proposed as a convenient parameter for estimation of antibiotic resistance.

WE097

Antibiotic residues in the polish coastal zone (southern Baltic Sea) - conclusions from 5 years study

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In the course of last decade pharmaceuticals have been recognized as relevant environmental contaminants. Special attention should be paid to antibiotic residues. These bioactive compounds are detected in low concentrations but their continuous input to the environment and prolonged exposure may strongly affect bacterial populations and induce biological effects in nontarget organisms, potentially disrupting ecosystem processes. Seas can be seen as the final sink of the most persistent antibiotic residues. This is particularly true for the Baltic Sea, due to its natural features and particularly large catchment area. In this 5 years study (2010-2014) the occurrence of 14 pharmaceuticals from sulfonamides, quinolones and tetracyclines groups was evaluated. Sediment and water samples were taken during r/v "Oceania" cruises from the southern Baltic Sea along the polish coast. The procedures for the analysis of target compounds applied solid-liquid extraction and tandem SPE technique for isolation, enrichment and clean-up and liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) with electrospray ionization for final quantitative and qualitative analysis. 14 compounds were identified in samples at concentration levels of ng?g-1 d.w. (sediment) and ng L^{-1}(water). The occurrence frequency differed significantly among the compounds and matrices. The most frequent compounds were sulfamethoxazole, oxytetracycline, trimethoprim and sulfachloropirydazine for sediment samples and trimethoprim, sulfamethoxazole and enrofloxacin for seawater samples.These compounds revealed also the highest concentrations in the collected samples. Occurrence of the identified antibiotics was characterized by high spatial and temporal variability. Laboratory studies to test the influence of antibiotics identified in the sediments on microbiological activity in the sediments were performed applying seven strains isolated from Baltic sediments. The results showed, that tested antibiotics, mainly tetracycline and mixtures of compounds, inhibited the growth of the bacteria. The results of chemical analyses indicate contamination of coastal zone by antibiotic residues and the results of biological tests indicate that these contaminants may affect sedimentary microbial community. Consequently, the studies should be continued to establish more extensive chemical and ecotoxicological databases on antibiotics and their degradation products in the Baltic Sea area.

WE098

PharmCycle: Sustainable Pharmaceuticals

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The key objective of the research project „PharmCycle" is to reduce prospective impacts of pharmaceuticals to the aquatic environment and drinking water supply. PharmCycle addresses therefore the life cycle of human pharmaceuticals, from the sustainable design and production, enhanced ecotoxicological risk assessment and improved wastewater treatment. The first cycle runs with antibiotics, which are selected from different prioritization lists due to their environmental concern. For these antibiotics an ecotoxicological risk assessment, integrating different chronic bacteria tests, is performed. A set of wastewater treatment pilot studies aims to reduce the concentration of the selected antibiotics in effluents. The second cycle runs with sustainable antibiotics, including newly designed antibiotic peptides, produced within PharmCycle using molecular and biotechnological methods. The third cycle runs with sustainable antibiotics designed with physical-chemical and in silico methods. Every cycle includes the upgraded ecotoxicologial risk assessment and investigations on wastewater treatment procedures. The approach of the project and first results are shown.

Pushing nanoparticle studies to the limit - working at environmentally relevant concentrations and with complex matrices (P)

WE099

Transport and toxicity for bacterial communities of reactive iron nanoparticles used for nanoremediation

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Highly toxic contaminants (e.g. As, chlorinated hydrocarbons, aso…) persist in the environment because of their low volatilization and/or hydrolysis and/or biodegradation. This situation is critical for public health and raises industrial and economic issues. Remediation techniques for these contaminants in groundwater are often technologically impossible, extremely expensive or hardly effective. Nanoparticles (e.g nZVI (Zero-Valent Iron), iron oxides, ferrate) applicable as in-situ reduction or oxidation agents for groundwater treatment show an extremely high reactivity leading to effective transformation of many contaminants into less toxic or benign products. However, these nanoparticles (NP) may also represent an additional contamination. Moreover, the different processes controlling the fate of NP and their toxicity towards microbial communities remain poorly understood. This study, part of the EU-project NanoRem (Taking Nanotechnological Remediation Processes from Lab Scale to End User Applications for the Restoration of a Clean Environment; EU FP7/2013-2017), aims to (i) study the mobility of NP in the presence or absence of biofilm by columns transport assays mimicking real aquifer conditions and (ii) to evaluate NP toxicity on bacterial communities (planktonic and biofilm grown onto sand). Results show that the mobility of nZVI widely depended on the water flow and/or NP concentration. The recovery at the column outlet was 1% for a 100 $m.d^{-1}$ and 40 % for 10 $m.d^{-1}$ water flow. The presence of biofilm in the column decreased its total porosity from 35% to 25%. Though the recoveries of nZVI in the presence or absence of biofilm were similar, the analysis of the sand showed that the variation of labile Fe content is concomitant with that of TOC, strongly suggesting NP-biofilm interactions. That was confirmed by CryoSEM images. Otherwise, measures of the denitrifying activity showed a toxicity of NP on planktonic bacteria starting at 50 mg $Fe.L^{-1}$ while cellular viability evaluated by Live and Dead method highlighted toxicity starting at 10 mg $Fe.L^{-1}$. It appears therefore that reactive NP, very useful for *in situ* groundwater treatment, can represent a source of emerging contamination. Other studies about mobility and toxicity of such particles will have to be conducted before a prospective large-scale application.

WE100

Nanoparticles, a risk for the environment through their transfer along an aquatic food chain?

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Despite the significant arise of nanomaterials application, the lack of knowledge about their toxicity can be detrimental for the ecosystem sustainability. Trophic transfer is often referred as an important pathway of nanoparticles contamination in aquatic ecosystems being reportedly the main exposure route to organisms. Among their great variety of nature and characteristics, gold nanoparticles (AuNPs, PEG coating, diameter 10nm) have been chosen as model contaminant due to their high stability in solution. This work aims to characterize AuNPs transfer, sequentially performed in controlled conditions, within three food chain levels. Thereby natural river biofilms contaminated in laboratory for 48h at low AuNPs' concentrations (of 10 and 100-fold lower than its LD_{50} for diatoms) were grazed by the fish *Hypostomus plecostomus* during a 21-days laboratory experiment. Analyses revealed that biofilms presented a high AuNPs retention capacity. Secondly, results point out that AuNPs were effectively transferred from natural biofilms to the grazer fish showing their ability to enter the food chain. Regarding AuNP fish distribution, organs involved in metabolism and excretion (liver and kidney) presented a significant bioaccumulation. Interestingly, AuNPs clearly penetrated the brain at the highest exposure concentration. Moreover, electronic microscopic observations showed tissue alterations indicating inflammatory responses. Following the concentrations used in the previous exposure essay, in a second phase, the predator *Anguilla anguilla* was fed with AuNP-spiked fish meat during a 21-days period. In reference to previous results, a transcriptomic approach was then focused in liver and brain tissues to evaluate the impacts on metabolic pathways.

WE101

Fate of TiO2 nanoparticles in the aquatic environment in the presence of anthropogenic compounds

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The increasing production and use of nanoparticles (NP) in consumer products inevitably lead to ENP emissions into the environment. The physicochemical properties of NP depend on various parameters (e.g. pH, cations, IS). In natural waters, the stability of NP can vary as a function of a sum of these parameters and occurs by one of the numerous scenarii. In particular, the presence of anthropogenic organic molecules (AOM) can change the NP fate. Also, the presence of NP may affect the organic pollutants (fate and toxicity). The main objective of the work was to study the aggregation of TiO_2 NP (pure hydrophilic 100 % rutile and pure hydrophilic 100 % anatase, 5−30 nm) in the presence of the most frequently occur and representative pesticides (glyphosate, AMPA, 2.4D) in natural waters considering lab experiments under relevant aqueous conditions (pH, ionic strength, presence and concentrations of mono- and bivalent cations). The presence of pesticides affected TiO_2 NP homoaggregation in solutions (IS=10^{-3}M - 10^{-2}M) with pH values below the NP point of zero charge (PZC) for the anatase NPs (pH=6.5) and with pH values above the NP PZC for the rutile NPs (pH=4.5). No changes in NP aggregation were observed in very low (IS=10^{-4}M) or very high (IS= 10^{-1}M) ionic strength solutions. The presence of the pesticides caused a significant modification of the NP surface charge (zeta potential) over a large range of salt concentrations (IS=10^{-4}M - 10^{-1}M). Compared to mono-valent cations (Na^+), bi-valent cations (Ca^{2+}) favor an increase in zeta potential of NP (anatase and rutile) at pH 8. There is no significant difference between at pH 5. Finally, these results demonstrated that, among the studied AOMs, glyphosate (with 4 pKa-s from 0.8 to 11) affects NP aggregation/stabilization in a wider range of physicochemical conditions. Overall, these results will aid in the evaluation of potential environmental risks posed by engineered NPs in the aquatic environments exposed to pesticide load.

WE102

Quantification of ionic silvers released from different surface-coated silver nanoparticles in the presence of fulvic acids

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Studying the roles of natural organic matter (NOM) in fate and transport of nanoparticles (NPs) after their releasing into the aquatic environment has been performed by many other researchers. Silver nanoparticles (AgNPs) were chosen for this study due to their potential toxic effects from both ionized and nanoparticulated forms. Especially, previous researches suggested that the toxic effects of AgNPs are mainly attributed to the silver ions (Ag^+) released from AgNPs. The objective of this study, therefore, is to assess the roles of NOM in dissolution for two AgNPs, differently coated with citrate (Cit) and branched polyethylenimine (BPEI). Pony Lake Fulvic Acid (PLFA) was selected as a model NOM due to its higher contents of S and N with high affinity to metallic Ag and Ag^+, compared to the other commercially available NOM standards. PLFA solutions were prepared in 10 mM $NaNO_3$ with the concentrations of 5 and 30 mg/L. Time-dependent dissolution of AgNPs was then monitored in the absence and presence of PLFA for 2 days and 6 days, respectively. Quantification of Ag^+ released from AgNPs for both cases was achieved by using an inductively coupled plasma mass spectrometer (ICP-MS). Prior to the analysis by ICP-MS, Ag^+ ions were separated from AgNPs particles using a ultrafiltration filter with 10 kD of molecular weight cutoff (MWCO) and a dialysis membrane with 0.5~1 kD of MWCO in the absence and presence of PLFA, respectively. As comparing to the case without fulvic acids (FA), significant decreasing of Ag^+ released from Cit-AgNPs was occurred in the presence of FA. This result may be from stronger interaction of Ag^+ with S and N of FA or shielding the active sites of NPs by FA. In contrast, Ag^+ released from BPEI-AgNPs was slightly increased in the presence of FA. Concentration of FA also played an important role in dissolution of AgNPs. For Cit-AgNPs, the amount of Ag^+ released in 5 mg/L of PLFA was 12.1 ± 2.16 μg/L, while the amount of Ag^+ released in 30 mg/L of PLFA was significant lower as showing of 2.62 ± 0.44 μg/L. The amount of Ag^+ released from BPEI-AgNPs in 30 mg/L of PLFA, 1.08 ± 0.19 μg/L, was also slightly lower than that in 5 mg/L of PLFA, 1.90 ± 0.02 μg/L. These lower releases of Ag^+ in higher concentration of FA indicate that higher FA may enhance the interaction of Ag^+ released from AgNPs with FA. Our dissolution research suggests that FA in the environment may reduce free Ag^+ and toxic effects of AgNPs to aquatic organisms.

WE103

Interference of signal transduction in zebrafish (Danio rerio) to zinc oxide nanoparticles

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Zinc oxide nanoparticles (ZnO NPs) are being utilized in an increasing number of fields and specialties (commercial applications). While the general toxicity or oxidative stress has been extensively studied, it is still unknown what toxicological pathway may occur following developmental stage. In this study, the developmental toxicity of ZnO NPs were investigated in the embryo-larval zebrafish, the transcriptional expression profiles by ZnO NPs was also investigated to ascertain novel genomic responses related to specific toxicity pathway. Zebrafish embryos were exposed to 0.01, 0.1, 1, and 10 mg/L ZnO NPs for 96 h post-fertilization (hpf). The ZnO NPs was found to exert a dose-dependent toxicity to zebrafish embryos and larvae, reducing the hatching rate and inducing malformation such as pericardial edema, yolk sac edema, and physical deformities. As the results of gene expression profiling using microarrays, total 1,586 genes were differentially up– or down–regulated (fold change > 2) in zebrafish followed by the exposure of ZnO NPs and $ZnSO_4$ (498 and 191 in ZnO NPs, and 681 and 216 $ZnSO_4$, respectively). Several genes that differentially regulated in ZnO NPs exposure shared similar biological pathways with those observed in $ZnSO_4$ exposure, but six genes (*aicda, cyb5d1, edar, intl2, ogfrl2* and *tnfsf13b*) were differentially regulated in ZnO NPs exposure, opposite or not in $ZnSO_4$ exposure. Real-time RT-PCR confirmed that the genes exposed ZnO NPs exposure was significantly changed comparing with the $ZnSO_4$ exposure. Our results are helpful to understand the mechanisms of ZnO NPs induced developmental toxicity in zebrafish.

WE104

Detection of metal-based nanoparticles in the river Dommel in the Netherlands

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We investigated the occurrence of inorganic nanoparticles in a natural\nsystem, the river Dommel in the Netherlands. The river itself is\nwell-studied as far as hydrology and water quality is concerned, easily\naccessible and one major wastewater treatment plant discharges onto\nthis river. We sampled the river water at various locations along the\nriver and collected samples of influent, effluent and sewage sludge\nfrom the treatment plant. The sampling campaign was carried in June\n2015 and these samples were analysed for seven metals (Ag, Au, Ce,\nLa, Ti, Zn and Zr) using inductively coupled plasma mass-spectrometry\n(ICP-MS), ultrafiltration and scanning electron microscopy (SEM).\n\nFrom the results we conclude that there are indeed nanoparticles\npresent in the system we studied, as we found titanium and gold\nparticles in the influent and effluent. The river water also contains\nnanoparticles, although they occur in clusters or attached to natural\ncolloids, so that the effective size is above 0.4 um. At most\n10 to 20% of the mass concentration is made up of free particles with\na size smaller than 20 nm or dissolved elements.\n\nWe found evidence that there is no appreciable anthropogenic emission\nof cerium into the river, based on the geochemical relationship\nbetween cerium and lanthanum. Besides, the effluent of the treatment\nplant has lower concentrations of the examined metals than the\nsurface water upstream. The treatment plant discharges much less of\nthese metals than estimated using previous publications. However, a\npotential source of titanium dioxide nanoparticles is their use\nin coatings of exterior surfaces, as the concentration of titanium\nincreased considerably in the urbanised area of the river Dommel.

WE105

Engineered nanoparticles in cosmetics according to EU 2011/696/EU: sample preparation and analysis

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The applicability of a previously developed generic scheme for detection, characterization, and quantification of engineered nanoparticles (ENPs) in a complex matrix has been extended to real product samples containing unknown particles. The extended generic multi-step sample preparation procedure includes: 0) pre-characterization of the sample, I) homogenization of the sample, II) ENP separation from the matrix, III) ENP enrichment, and IV) ENP stabilization. In this study, sunscreen, as a common example of complex matrix, which potentially contains TiO_2 as UV-filter was selected to apply the proposed extension of generic sample preparation approach. Pre-characterization in order to identify possibly present ENPs was designed and applied to the sunscreen sample. We demonstrated that the extended generic sample preparation scheme is valid and pre-characterization of the sample is a useful tool to isolate target ENPs for further analysis. TiO_2 ENPs present in the sunscreen could be isolated by a combination of ultra-centrifugation and hexane washing with sufficiently high recoveries for performing further analysis on the particle size. To apply EU 2011/696/EU recommendation where materials are classified as nanomaterials based on number-based particle size distributions, the size distribution of the isolated TiO_2 ENPs was determined by asymmetric flow field-flow fractionation (AF^4) coupled to multi-angle laser light scattering (MALLS) and inductively-coupled plasma mass spectrometry (ICP-MS). AF[4]-MALLS-ICPMS analysis indicated only a slight shift of the size distribution towards larger diameters. Therefore the mass-based size distribution, which was derived from AF[4]-ICPMS analysis, was converted into a number based size distribution. The applied conversion algorithms are very sensitive towards mass-based signals of small particle sizes. Consequentially high signal noise levels for small particles potentially introduce large errors in the number-based ENP size distribution. *Acknowledgment* – The NanoDefine project has received funding from the European Union's Seventh Programme for Research, Technological Development and Demonstration under grant agreement No 604347-2.

WE106

Pitfalls and challenges of microplastic analysis with Raman micro-spectroscopy

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Microplastic residues both in marine and freshwater systems have recently been a topic discussed in the public and in the scientific community. For freshwater systems, available information is still scarce. An increasing number of studies reports toxicological and ecotoxicological effects of microplastics. Particles with a diameter < 300 μm are supposed to be very important and interesting for toxicological and ecotoxicological effects. A major problem is the lack of standardised methods for sampling and analysis of microplastics. Many studies so far published relied even on the naked eye to identify particles as polymers instead of chemical identification. Toxicological effects may be related to particle size, shape, material and particle concentration. A very promising approach to detect very small particles and to achieve the latter information is the use of Raman micro-spectroscopy which provides a high spatial resolution of 1 μm. First, the focus was put on aqueous matrices and the extraction and detection procedure. An example of recently not investigated sources for microplastic particles could be slow or controlled-release fertilizers coated with polymer resins and super absorbers, both used in forestry, agricultural and horticultural applications. The latter could be transferred to surface waters or groundwater. Due to the novelty of the subject, this study provides some information on potential pitfalls and challenges of microplastic analysis.

Ecological traps for wildlife driven by pollutants (P)

WE107

Heavy metals, oxidative stress and immune function in common moorhens from natural and artificial wetlands

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The use of effluent from wastewater treatment plants for maintenance of wetlands is becoming common practice in semiarid regions. We suggest that this type of ecosystem could act as an ecological trap, because it concentrates pathogenic organisms and chemical pollutants coming from several sources. Heavy metals have been reported as a cause of lethal poisoning and sublethal effects by impairing biochemical processes. This study determined levels of heavy metal in waterbirds from a wetland that receive effluent from a wastewater treatment plant (Navaseca Lake) and how these pollutants alter oxidative stress biomarkers and the immune function in comparison to birds from non-polluted sites (Tablas de Daimiel National Park, TDNP) in Central Spain. Blood samples were collected from 127 common moorhens (*Gallinula chloropus*) to determine levels of As, Cd, Mn, Hg, Pb, Se, Cu and Zn, oxidative stress biomarkers and immune function parameters. Mn, Hg and Se were significantly higher in moorhens from TDNP. These recent findings are in contrast to our previous work at these sites in which we found higher PCB levels in birds from Navaseca Lake. Moorhens from TDNP displayed higher levels of retinol, α-tocopherol, MDA and SOD activity. Moorhens from Navaseca showed higher bactericidal activity in blood and higher haptoglobin levels in plasma and slightly lower cellular response to phytohaemagglutinin injection. Hg and Se levels were positively correlated ($r=0.410$, $p< 0.001$). Hg and Se were positively correlated with SOD activity and negatively correlated with GPX and bactericidal activity. Moorhens from Navaseca Lake have shown higher levels of some POPs, but lower levels of heavy metals compared with birds from TDNP. Additionally, evidence of increased oxidative stress and capacity to cope with pathogens, as shown by the elevated bactericidal activity and haptoglobin levels was found. These results may indicate that moorhens from Navaseca Lake are at greater risk of exposure to some organic pollutants and a wider range of pathogenic bacteria than at TDNP. This could explain the overexpressed immune response and the evidences of oxidative stress. That the highest levels of heavy metals were found in moorhens from TDNP may show the highest accumulation of these pollutants in this floodplain. Navaseca Lake, as a eutrophic wetland, attracts high numbers of waterbirds as feeding area, but it may act as an ecological trap due to biotic and abiotic threats to birds.

WE108

Does food quality and quantity, limited by parental feeding strategies, influence offspring fitness? A case study in Lesser black-backed gulls (Larus fuscus)

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Exposure of free-ranging bird populations to varying environmental conditions (e.g. contamination and food availability) are factors with potential to modify individual fitness at different developmental stages by triggering changes in normal physiological processes (e.g. enzymatic functions). The Lesser Black-backed Gull (*Larus fuscus*) is a seabird species with a high individual variation in foraging strategies and an ability to thrive in urban landscapes. Over the last decades, these gull species foraging habits have become increasingly dependent on human activities – and food resources- which in return increases susceptibility of exposure to toxic substances. The ingestion of contaminated food may be then transferred from adult birds to their offspring via parental care during chick rearing. The goal of this study was to address how a shift in foraging behaviour of *L. fuscus* adults, during chick development, and coupled with food quantity provided may interact with chick fitness during breeding. In order to assess this topic, during 2016 breeding season, *L. fuscus* eggs were collected in Oostende harbour (first or second egg collected). Eggs were only collected at sites where the natural hatching and survival rates are known to be very low, and were then moved to specifically designed aviaries (VOC Oostende). There eggs were bred in RCOM 20 SURO incubators and chicks hand-raised for 30 days. After hatching, individuals were randomly assigned to 3 different diet treatments: a predominantly marine (80% marine: 20% terrestrial), predominantly terrestrial (80% terrestrial: 20% marine) or mixed (50% marine: 50% terrestrial) diet, and food was provided ad libitum. In addition, two other additional diets were tested in which daily energetic input was reduced to comply with minimum energetic requirements: marine restricted or terrestrial restricted. Following this, chicks' were measured every 2-3 days and blood samples collected (days 12, 21 and 30) for posterior assessment of mercury burden, lipid peroxidation and activity of a battery of enzymatic markers (cholinesterase, lactate dehydrogenase, catalase and glutathione-*S*-transferase). Mercury burdens was typically higher in chicks fed a predominantly marine diet. Furthermore, differences in enzymatic activities were observed in chicks between diets and varying with age, corroborating that both quality and quantity of food provided may interact with early chick fitness.

WE109

Persistent organic pollutants (POPs) in Pontoporia blainvillei from Franciscana Management Area II - Southeastern coast of Brazil: assessment of population structure and temporal trend

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Persistent organic pollutants (POPs), such as polychlorinated biphenyls (PCBs) and dichlorodiphenyltrichloroethane (DDTs), are synthetic compounds that bioaccumulate in fat tissues and biomagnify through the food web due to their physicochemical properties. Marine mammals have been proposed as sentinels of marine environmental health, since these organisms are top predators and may accumulate high concentrations of organic pollutants. Franciscana dolphin (*Pontoporia blainvillei*) is a small cetacean found exclusively in the Southwest Atlantic Ocean and is the most endangered dolphin in the area. Recently the four Franciscana Management Areas (FMAs I to IV), initially proposed to improve the management of the species, were revised. In order to contribute to these FMAs, persistent organic pollutants were analyzed in 101 blubber samples of *Pontoporia blainvillei* collected in the FMA II corresponding to the coast of the state of São Paulo (São Paulo North, SPN, São Paulo Center, SPC, and São Paulo South, SPS). POPs distribution were similar among the three Franciscana stocks with a predominance of PCBs (286 – 22,823 ng g^{-1} lipid weight, lw) and DDTs (39 – 7,186 ng g^{-1} lw). The population from FMA II - SPC presented the highest values. This was expected since in this area a major industrial complex and the largest harbor in South America are located. The three Franciscana stocks showed similar profiles of PCB congeners, with predominance of hexa-chlorinated compounds (mainly PCB-153 and PCB-138), representing approximately 48% of the total PCB amount. Significant differences were observed between juveniles and adults, while among sex there is no significant difference except for DDTs, which were detected in relatively higher concentrations in males. The PCB/DDT ratio showed similarity between SPN and SPC populations; however they were significantly different from SPS ($p < 0.05$). Assessment of PCB and DDT in Franciscana dolphins from FMA II in the last 15 years (2000 – 2014) suggests a slight decline in their concentrations. POPs data support the subdivision of FMAII, especially the southern area (i.e. SPS) that could be considered as a different Management Unit for future conservation plans on the species.

Fish model species in environmental toxicology (P)

WE110

Cross-species extrapolation of uptake and disposition of neutral organic chemicals in fish using a multi-species physiologically based toxicokinetic model

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The potential to bioaccumulate is generally considered an unwanted substance property. Consequently, chemical legislations, such as the European REACH regulation, require the chemical industry to provide bioconcentration data for chemicals that are produced or imported at volumes exceeding 100 tons *per annuum,* or if there is concern that a substance is persistent, bioaccumulative and toxic. To fill the existing data gap for chemicals below this stipulated volume without the need for additional animal experiments, physiologically based toxicokinetic (PBTK) models can be used to kinetically predict whole-body bioconcentration as well as internal concentrations of neutral organic chemicals in different fish tissues. PBTK models have been developed for many different fish species with promising results. In this study, we developed PBTK models for zebrafish (*Danio rerio*) and roach (*Rutilus rutilus*) and combined them with existing models for rainbow trout (*Onchorhynchus mykiss*), lake trout (*Salvelinus namaycush*) and fathead minnow (*Pimephales promelas*). The resulting multi-species model allows for cross-species extrapolation of the bioaccumulation potential of neutral organic compounds. Predictions were compared with experimental data and were accurate for most substances. Our model holds strong potential for the probabilistic risk assessment of a chemical's bioaccumulation potential with special emphasis on cross-species extrapolation of sensitivity.

WE111

Fish Metabolism Studies

G. Gonsior,

Stagnating growth in global food production is leading to an increased focus on alternative food resources. The global aquaculture industry continues to expand since fisheries have to deal with decreases in fish stocks and climatic effects. Fresh water fish farming is one of the most productive sectors for this industry. This leads to the problem: How to feed the fish? High quality food might be needed as a resource for humans. Therefore the risk is high that pesticide contaminated food will be fed to animals like fish. In most cases these residues will be metabolised. Therefore it is essential to identify metabolites which have a potential risk for human health. So far no specific guideline for fish metabolism is available. A testing approach will be presented with the focus on lab testing.

WE112

Investigations on biodiversity and ecosystem health of the river Nidda within the framework of the project 'NiddaMan'

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With almost 100 km length and a catchment of 2000 km², the river Nidda represents a great number of European streams. Being in its natural state near the spring in the Vogelsberg Mountains in Upper Hesse (Germany), the river Nidda rapidly changes its chemical and biological water status due to anthropogenic influences along its course. Discharges of local wastewater treatment plants, diffuse emissions of agricultural substances and substance inputs from tributaries as well as structural alterations are affecting the river on its way downstream, until entering the river Main near Frankfurt (Germany). The 'biodiversity and ecosystem health part' of the BMBF-project 'NiddaMan' investigates the ecological importance of diverse stress factors affecting fish in the Nidda catchment area. In an active monitoring approach, rainbow trout (*Oncorhynchus mykiss*) are directly exposed in cages to the water of the Nidda. Furthermore, feral fish are investigated at different sites in the catchment area with the aim to validate these responses obtained by active monitoring .To identify the influence of different input sources, like wastewater treatment plants, a local mineral spa and a papermill, samples from representative sites are also chemically analysed , and in addition, biotests addressing genotoxic- (micronucleus assay), cytotoxic- (histopathology), embryotoxic- (DarT) and dioxin-like (EROD assay) effects are conducted. The overall aims of this project part are the development of strategies to counteract water quality impairment induced by environmental stressors and to support river ecosystem recovery. Acknowledgement: The project NiddaMan is funded by the Federal Ministry for Education and Research (BMBF) within the ReWaM initiative under project code 02WRM1367.

WE113

Pollution induced effects in feral fish from the river Holtemme assessed by toxicogenomic techniques

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Anthropogenic and natural pollutants often result in adverse effects on different levels of biological organization. The aim of this project was the linkage of biochemical and physiological responses with parameters of demographic relevance (survival, growth, reproduction) by the use of toxicogenomic techniques. Deep sequencing technologies of the whole transcriptome enables the investigation of the specific mechanisms by which stressors such as xenobiotics can interact with molecular toxicity pathways. Therefore, a wide range of potential impacts of complex chemical mixtures can be examined. In this study, samples of wild brown trout (*Salmo trutta)* from the river Holtemme (Germany) were collected in a sampling campaign by the RWTH Aachen University and Helmholtz Centre for Environmental Research (UFZ) in 2014. This river is of particular scientific interest due to its specific point sources of pollution. Therefore, four sampling sites were chosen to determine effects of agricultural use or discharges of waste water treatment plant (WWTP) effluents. Hepatic tissues of fish samples were used for RNA-sequencing-by-synthesis technologies in cooperation with the Toxicology Centre of the University of Saskatchewan (Canada). The first functional pathway analysis of the expression data shows more than 600 significantly altered genes at study sites downstream of WWTP effluents and agriculturally used area, respectively. Earlier studies detected pollution induced/inhibited effects on micronucleus formation, biotransformation enzyme activity, biomarker of exposure and oxidative stress in the same fish samples from the river Holtemme. This reveals that multiple known toxicity pathways have been altered, including changes of phase I metabolism enzymes expression or steroid biosynthesis. Further analysis should investigate pollution effects and specific mechanisms of actions with the ultimate goal to predict potential adverse outcomes as part of the environmental risk assessment of chemicals and to discover new biomarkers for ecotoxicological application.

WE114

Biomarkers measured in situ using native fish in three agricultural areas associated with environmentally valuable wetlands in Costa Rica

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Indigenous fish have been used to apply a biomarker approach in environmental evaluations in Costa Rica,. Caging experiments using the species *Parachromis dovii* have been carried out along environments exposed to different agricultural activities. *In situ* 48-hour exposures of caged *P. dovii* were performed at three different areas of the country, characterized by agricultural crops associated with wetlands: one at the North-Pacific, close to the Palo Verde lagoon (Palo Verde National Park) four sites where studied in this area where mainly rice and sugarcane are grown. A second area is located at the North zone, upstream of the Refugio de Vida Silvestre Caño Negro (RAMSAR) four sites were sampled there, where the main crop is pineapple. The third zone is on the Caribbean side of the country, six sites were sampled on a region characterized by banana, rice and pineapple plantations, upstream from an estuarine lagoon (Laguna Madre de Dios). A set of biomarkers: cholinesterase (ChE), glutathione S-transferase (GST) and catalase (CAT) activities; as well as lipid peroxidation (LPO) were measured in fish exposed *in situ*. Pesticide residue analyses were performed for every fish deployment experiment and relationship between pesticide pollution and biomarker responses was evaluated. Pesticides were present in all three studied areas. In Caño Negro, residues were only detected in one of the four sampled sites. The other two areas had different pesticide concentrations in all sampling sites. Biotransformation and oxidative stress-related biomarkers (GST, CAT and LPO) showed clear differences at one of the Palo Verde sites, where several pesticides were detected in water. ChE was inhibited at two Caño Negro sites but pesticides were only detected in one of them. In the Madre de Dios area, all four biomarkers

showed significant changes at different sites, agreeing with the presence of pesticides measured in water samples. Data suggest that biomarkers measured in caged *P. dovii* can give information on early effects caused by exposure to pesticides as significant changes were observed at most impacted sites. Regarding the areas and crops assessed, some differences in environmental conditions and agricultural practices might be enhancing runoff from agricultural areas in the Caribbean zone compared to the other two areas.

WE115

Effects of Long- term exposure of different intensities magnetic field on biochemical and enzymatic parameters in Acipenser ruthenus

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Considerable attention is focused on the effects of electromagnetic field (EMF) due to its wide-ranging use in everyday life. Anthropogenic electromagnetic fields were induced to the aquatic environment by different sources such as underwater power and telecommunication cables, electrical heating cables for oil and gas pipelines as well as generating units and submerged substations. It is known that magnetic field can change cell behaviors and activation by affecting the biochemical and/or biophysical process in aquatic animals. In this study, the effects of variable magnetic fields on biochemical factors of Acipenser ruthenus were investigated. 14 juvenile fish was exposed to magnetic fields with different intensities (3, 5, 15, 25 mt) induced by electrical power supply for 2 months. The results showed that there was not significance difference in growth rate between the control and exposed fish. However, there were significant differences in lysozyme level and complement activity between control and exposed fishes.

WE116

Sublethal effects of copper in early life stages of development of rainbow trout (Oncorhynchus mykiss)

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Copper (Cu) is an essential element needed in small quantities for organism metabolism as well as for functioning of some essential enzymes. Due to its fungicidal properties, it has been extensively used for vineyard treatment against plagues resulting in its high emission into soils and aquatic environments. At high concentrations, copper can inhibit growth and interfere in several cellular processes including respiration, synthesis of proteins and cellular division. The aim of this work was to study the possible sublethal effects that copper could cause in the early life-stages of development of rainbow trout fish (*Oncorhynchus mykiss*). Eyed-stage embryos rainbow trout (265 °J) were exposed in semi-static conditions to sublethal concentrations of $CuSO_4$ up to the larval stage (528 °J) under laboratory-controlled conditions. During 23 days, they were exposed to the environmentally-realistic concentration of 2 μg/L Cu^{2+} and to a 10 fold higherconcentration 20 μg/L Cu^{2+}. Several biological and morphological (survival, hatching success, malformation, growth), functional endpoints (swimming activity) and genotoxicity (DNA damage) were studied. Exposure to 20 μg/L Cu^{2+} resulted in a significant reduction in hatchability (63%) and an increase of half-hatched embryos (25%). Copper had an inhibitory effect on hatching. At the end of the exposure, no significant differences were observed in growth of the larvae exposed to the highest Cu concentration. However, larvae exposed to the weakest Cu concentration resulted with a major growth in comparison to the control. Malformations were recorded in larvae of 492 °J, and the percentage of malformed larvae was significantly higher for the conditions of 2 and 20 μg/L Cu^{2+} (with 53 and 60% of malformations respectively) in comparison to the control (27% of malformation). For both conditions, the skeletal malformations were the most observed. Behavioural study was conducted at the end of the experience by imaging analysis system Daniovision (version 10.0 of Noldus). Larvae exposed to the weakest copper concentration were less mobile, whereas no significant differences were observed in total distance travelled by the larvae exposed to the strongest concentration of copper. A comet assay was done and no evidence of significant DNA damage was observed for both conditions. This study confirms the toxic effects of copper on early life-stage of rainbow trout, even at the lowest environmentally relevant tested concentration.

WE117

Zinc or swim: are mechanisms of sub-lethal zinc toxicity conserved in a key southern hemisphere fish species?

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Metal contaminants have been shown to cause sublethal toxicity in model fish species. However, very little is known regarding the sensitivity of "non-model" fish, particularly indigenous species in the Southern hemisphere. This study investigated the impacts of zinc, a ubiquitous metal contaminant, on the freshwater fish, inanga (*Galaxias maculatus*). Inanga is widespread throughout the Southern hemisphere, is a frequent inhabitant of near-coastal streams impacted by pollution, and is of significant cultural and economic importance. Inanga also have a number of unusual physiological characteristics, including extensive use of the skin as a transport surface, and an amphidromous life-history that conveys an exceptional ionoregulatory ability. These factors may alter the relationship between metal exposure, accumulation and impact, particularly for metals such as zinc, which have impacts on epithelial processes. To determine mechanisms of sublethal toxicity, fish were exposed to graded, environmentally-relevant concentrations of zinc over 96 h (1000, 270 10, 0 µg L^{-1}). Endpoints measured included those relating to ionoregulatory disturbance (whole body calcium and sodium influx), respiratory/metabolic impairment (respirometry) and oxidative stress (catalase activity and lipid peroxidation). Tissue accumulation of zinc was also measured. Results indicated that zinc exposure stimulated catalase activity, but also increased hepatic lipid peroxidation. Effects were also seen on ion transport, with an impairment of calcium influx, consistent with other studied fish, but a stimulation of sodium influx. However, these effects were only observed at the highest exposure concentration (1000 µg L^{-1}). No effects on metabolic rate were observed, and whole body zinc was homeostatically-regulated beyond the lowest zinc exposure level. Overall, these data suggest some similarities in terms of inanga response to zinc relative to model fish, but also some key differences (increase in sodium influx). These data were the first to examine the sensitivity of juvenile galaxiid fish to zinc, information that will be critical to ensuring adequate environmental protection of this important species.

WE118

Recovery of seahorse (Hippocampus reidi) after acute and subchronic exposure to water soluble fraction of diesel oil using a DNA damage approach

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The main goal of this study was to investigate the effects of acute (12, 24, 48 and 96h) and subchronic (168 and 360h) exposition to 50 % water soluble fraction (WSF) of diesel oil on the integrity and functionality of DNA in seahorse *Hippocampus reidi* (Ginsburg, 1933). In addition, we investigated the ability of fish to recover (504h without contamination) after 168h of exposure to the above WSF using the same DNA endpoints. The comet assay and the micronucleus test were used in peripheral blood erythrocytes of 112 specimens (16 fish per condition/time: 8 for WSF exposure and 8 for its respective control group; and 16 for recovery experiment: 8 for post-exposure period and 8 for it control group). Our results revealed significant increases in the frequencies of DNA strand breaks and micronuclei frequency with increasing exposure period and thus demonstrated the genotoxic and mutagenic potential of this contaminant on fish. Furthermore, both techniques detected the highest DNA damage at 168 h of exposure, followed by statistically decline at 360 h of exposure. During the recovery experiment, DNA strand breaks and micronuclei frequency were significantly reduced after 504h post-exposure, when compared with 168h exposure group. The results supported the integrated use of comet assay and micronuclei test in determining the early impact and recovery from acute and subchronic exposure caused by diesel oil contamination. Besides, *H. reidi* proved to be a useful bioindicator in the determination of diesel spill impact on fish populations. Financial support: FAPES, UVV.

WE119

Effects of four substituted phenylamine anti-oxidants (SPAs) in chronic fathead minnow early life stage tests

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Substituted phenylamine antioxidants (SPAs) are a group of chemicals on Canada's Domestic Substances List. They are high volume chemicals with a variety of uses, including: dye formulations, photosensitizers, lubricant antioxidants, viscosity improvers, and dispersants. SPAs are considered to be persistent, bioaccumulative, and inherently toxic. They can enter the environment in municipal wastewater effluents or via direct run-off from road surfaces. We investigated the chronic toxicity of four SPAs in a 21-day fathead minnow embryo-larval assay. Embryo-larval fathead minnow were exposed to SPAs in sediment because of SPA's affinity for organic matter. Reference sediment (2 % organic carbon) was spiked with 125, 250, 500, or 1000 μg/g of each SPA, and left for one month to equilibrate prior to fish exposures. Fathead minnow embryos and larvae were exposed in beakers with 35 g sediment and 700 mL lab water, with sediments and water renewed daily. Glass mesh-bottomed cups prevented direct contact of embryos and larval fish with sediment, and facilitated daily transfer to new exposure beakers. Endpoints were % survival to hatch, hatching success, deformities at hatch, survival til 8 and 16 days post-hatch (dph), and weight, length, and condition factor of larvae at 8 and 16 dph. All four SPAs decreased larval survival, with LC50s in the range of 250-350 μg SPA/g sediment. Water and sediment concentrations of SPAs are being measured to verify fish exposure concentrations. Concentrations of SPAs in sediment that affected survival of embryo-larval fish will be compared to measured concentrations of SPAs in biosolids and wastewaters from municipal wastewater treatment facilities in Canada.

WE120

Accumulation and maternal transfer of 2,2',4,4'-tetrabromodiphenyl ether (BDE-47) from an oral exposure in medaka fish (Oryzias latipes)

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In aquatic species, dietary exposure is the most environmentally realistic exposure route to contaminants with very low water solubility. Previous investigations conducted in our laboratory with post-hatch early developmental stages of medaka (*Oryzias latipes*) showed the capacity of 2,2',4,4'-tetrabromodiphenyl ether (BDE-47) to accumulate progressively during a 40-d oral exposure but without disturbing normal growth. Now, in this study effects assessment on reproductive capacity (i.e., fecundity and fertility) and BDE-47 accumulation were measured in adults and in resultant embryos and larvae from a 40-d maternal and paternal dietary exposure. Sexually mature medakas (165-d old) on a 3:2 male to female ratio were fed for 40 d with a diet containing 1000 ng BDE-47/g or with a control diet without BDE-47. At predefined time points (i.e. after 10, 20, 30 and 40 d since the start of the exposure), males and females were sampled and the eggs laid the same day were collected for a BDE-47 quantitative analysis. In addition, the reproductive capacity was assessed for the same time points and the resultant eleutheroembryos analyzed for BDE-47 content. The BDE-47 detected in embryos and eleutheroembryos ranged from 200 to 500 ng/g wet weight from 10 to 40 exposure days, respectively with levels comparable in embryos and eleutheroembryos. In the parents, the BDE-47 concentration was comparatively higher in males with respect to females. These differences correlated with the higher lipid content quantified in males. No significant effects on reproductive capacity were observed. This work was made possible by Spanish Government Grants CTM2013-44986-R and CTM2014-52388-R.

WE121

The evaluation of betamethasone as an endocrine disruptor using a fish model

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To date, little-to-no data have been collected on synthetic glucocorticosteroids in the environment and their potential endocrine disruption (ED) activity. They are antagonists to estrogen, but are expected to be found in higher concentrations in the environment due to higher therapeutic doses and a larger patient population. Steroid hormones have the potential to interact with the hypothalamic-pituitary-gonadal (HPG) axis and disruptions of this pathway can result in decreased reproduction and/or adverse developmental effects in offspring. Betamethasone, a synthetic glucocorticosteroid, has been on the market in the United States since 1983, and is on the WHO Model List of Essential Medicines. It mimics the action of cortisol and may disrupt the HPG axis. Studying fish for the ED potential of betamethasone is logical, as they could be exposed to pharmaceuticals in waste water treatment plant effluent following normal patient use and excretion. Additionally, fish have a much shorter time to reproduction and overall lifespan, making them ideal for determining lifetime, low-level chronic effects. Environmental modelling was used to estimate betamethasone concentrations in surface water and then environmentally relevant concentrations were then used in a two generation fish full life cycle (FFLC) study with Japanese medaka. Gross endpoints, as well as VTG and gonadotropin effects were evaluated in a tiered fashion.

WE122

Use of molecular and hormonal factors as biomarkers of Bisphenol A in Yellowfin Seabream (Acanthopagrus latus)

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Abstract Bisphenol A [BPA; 2,2-bis(4-hydroxyphenyl) propane] is an industrial important chemical that is abundantly used as a primary raw material for the production of plastics and resins. This substance is one of the most well-known endocrine disrupters widely released in aquatic environments mainly through urban and industrial sewage effluents; thereby posing a potential threat to the organisms in the ecosystems. In the present study, effect of BPA on liver DNA integrity, erythrocytic nuclear abnormalities (ENA) stimulus and thyroid hormone balance in male yellowfin seabream (Acanthopagrus latus) were investigated. For this reason, fish received intraperitoneal injections during a period of 2 weeks with 10, 50, 100 and 150 ug g-1 week-1 of BPA. Our results demonstrated a significant increase in fish micronuclei frequency after treatment with bisphenol A. In addition, the rate of liver DNA integrity was tested using the DNA alkaline unwinding assay. Results showed a decline in the rate of liver DNA integrity after 7 and 14 days of BPA exposure in comparison to the control group. In BPA treated fish, hepatic somatic index (HSI) rose in a dose-dependent manner ($R2 = 0.96$) after 7 days of treatment. Our results indicated significant reduction in plasma triiodothyronine (T3) and high levels of thyroxine (T4) in treated fish comparing the control groups. The findings of the project revealed that BPA strongly affected DNA and caused considerable breaks in strand of the living molecule. Additionally, erythrocytes are adversely damaged by BPA which is studied as ENA. Hence, it could be concluded that Micronucleus test and DNA strand breaks are sensitive cellular and molecular biomarker of BPA. Keywords: Acanthopagrus latus, Biomarker, Bisphenol A, Micronucleus test, DNA integrity, Endocrine disrupters, Thyroid hormones

WE123

The Effects of mixtures of androgenic and estrogenic chemicals on adult Murray River Rainbowfish

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When individual organisms and populations are exposed to Endocrine Disrupting Chemicals, it is likely that they are exposed to complex mixtures rather than to single chemicals. Studying the effects of single chemicals may not reflect the multitude of antagonistic or synergistic stimuli to which wildlife are subjected. A common source of both androgenic and estrogenic steroid hormones to the environment is the runoff resulting from animal feedlots. This is mainly due to the extensive use of anabolic implants in the beef industry. Implants have been used for more than 50 years due to their ability to increase the body protein content, growth efficiency and carcass weight and contain estrogens such as oestradiol (E2), progestins as progesterone and androgens such as trenbolone (TBL) acetate as ingredients. In the blood of livestock, administered trenbolone acetate is hydrolysed to 17β trenbolone and this by epimerization is then converted to 17 α trenbolone. Despite 17β trenbolone being a potent androgen receptor agonist with a 10 fold higher binding affinity, 17α trenbolone comprises about 95% of the total excreted product. The aim of this study was to evaluate the effects of 17α trenbolone alone and in combination with two endocrine disruptors of different modes of action, i.e. 17β oestradiol (50 ng/L) and Fadrozole (250 μg/L) on adult rainbowfish (*Melanotaenia fluviatilis*). The relative gene expression of steroid receptors in the liver as well as induction of vitellogenin in the plasma were used to assess potential effects. Results show that the expression of ERα and ERβ were significantly induced after exposures to TBL + E2 at both low (5ng/L) and high concentrations (500 ng/L) of 17 α trenbolone. The ERα expression in the TBL + E2 exposures correlated with the hepatic Vtg mRNA. The expression level of the ARβ was significantly reduced in the liver of *M. fluviatilis* when exposed to low concentrations of TBL in combination with E2 compared with controls, whereas when combined with high concentrations of TBL there were no significant differences. Vitellogenin induction was twofold lower when fish were exposed to E2 with a high doses of TBL than when E2 was mixed with a low dose of TBL. These findings demonstrate that E2 may have anti androgenic effects, leading to the down regulation of ARβ. In the aquatic environment where mixtures of androgens and estrogens are known to occur, similar counteracting effects are likely to occur.

WE124

Developmental effects of 2 and 6-hydroxychrysene on zebrafish embryos

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Chrysene is one of most persistent polycyclic aromatic hydrocarbons (PAHs) present in crude oil. In the water column, chrysene likely undergoes photochemical oxidation potentially forming oxygenated photoproducts such as 2-hydroxychrysene (2-OH-chry) and 6-hydroxychrysene (6-OH-chry) in the water column. Previous *in vitro* studies showed that 6-OH-chry was an estrogen receptor (ER) antagonist, but 2-OH-chry was an ER agonist. Since dysregulation of estrogen pathways can cause abnormal fish development, the objectives of this study were to investigate the toxicity of chrysene photoproducts on embryonic development. For this purpose, *Danio rerio* embryos (2 hpf) were treated at several concentrations of 6-OH-chry (0.5-8 μM) and 2-OH-chry (0.5-5 μM) to determine percent survival and the occurrence of deformities at 76hpf. Survival after exposure to 2-OH-chry was not affected; however, treatment with 6-OH-chry significantly decreased survival. The LC50 value for 6-OH-chry was ±1.14 μM for a 74hr exposure. Significant differences in the occurrence of cardiac abnormalities were also observed. The EC50s for 6-OH-chry and 2-OH-chry were ±1.3 and ±0.64 μM, respectively. Despite the regio-selective differences in toxicity that was observed, co-exposure to an ER antagonist (ICI 182,780) or G-Coupled Estrogen Receptor (GPER) antagonist (G-15) did not affect the mortality and deformity percentages. Similarly, mRNA of GPER, or downstream transcription factors (Hand2; Lrrc10) was unaltered by hydroxyl-chrysene exposures. Since cardiac abnormalities due to PAHs are mediated through the Aryl hydrocarbon receptor (AhR), each compound was evaluated using an AhR transactivation bioassay, and neither compound showed AhR activity. Evaluation of embryos post-exposure indicated concentrations of 2-OH-chry, but not 6-OH-chry, in the yolk sac in a dose-dependent manner suggesting a potential regio-selective difference in the uptake or metabolism for each compound. Our findings indicate developmental toxicities of hydroxylated chrysenes may not be mediated through AhR or ER, but may be due to differences in embryonic clearance mechanisms.

WE125

ASSESSMENT OF THE RESPONSE ZEBRAFISH EMBRYOS TO ANALGESICS

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The pain medications are products that are consumed in large quantities worldwide. These drugs can cause harmful effects on aquatic organisms because they are designed to have a physiological effect at very low concentrations. The aim of this study was to determine the toxic effect of 6 types of analgesics: Acetylsalicylic acid, Ibuprofen, Diclofenac, Naproxen, Paracetamol and mixture of Naproxen and Paracetamol in zebrafish embryos, to evaluate their sensitivity. Static bioassays were performed with a duration of 48 hours, the embryos were exposed to 6 concentrations of drugs: 100, 75, 50, 25, 12.5 and 6.25 mg L^{-1} (12 replicates), plus a control without toxic. In the tests LC_{50} was determined and the degree of lipid peroxidation (TBARS) was evaluated. The toxicity of analgesics was (from highest to lowest toxicity): Diclofenac> Paracetamol> ibuprofen> mixture Naproxen and Paracetamol> naproxen> Acetylsalicylic Acid. The degree of lipid peroxidation was higher in embryos exposed to Paracetamol (83 nM MDA mg^{-1}) and the lowest in those exposed to acetilsalisilico acid (24.3 nM MDA mg^{-1}). Organisms exposed to sublethal concentrations (LC_{25} LC_{15}) during the recovery period, showed higher degrees of lipid peroxidation to that observed in control group at the start of the test, the majority of the embryos failed to hatch and died between the fourth to the seventh day.

WE126

Chronic exposure to pentachlorophenol alters thyroid hormones in zebrafish

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Pentachlorophenol (PCP) is frequently detected in the aquatic environment and has been implicated as an endocrine disruptor in fish. Here, 4-month-old zebrafish(Daniorerio) were exposed to one of four concentrations of PCP (0.1, 1, 9 and 27 ?g/L) for 70 days. The effects of PCP exposure on plasma thyroid hormone levels, and the expression levels of selected genes were measured in the brain and liver. PCP exposure at 27 ?g/L resulted in elevated plasma thyroxine concentrations in male and female zebrafish, and depressed 3, 5, 3?-triiodothyronine concentrations in males only. In both sexes, PCP exposure resulted in decreased mRNA expression levels of thyroid stimulating hormone ?-subunit (tsh?) and thyroid hormone receptor ? (tr?) in the brain, as well as increased liver levels of uridinediphosphoglucuronosyltransferase(ugt1ab) and decreased deiodinase 1 (dio1). We also identified several sex-specific effects of PCP exposure, including changes in mRNA levels for deiodinase 2 (dio2), cytosolicsulfotransferase (sult1 st5), and transthyretin(ttr) genes in the liver. Environmental PCP exposure also caused an increased malformation rate in offspring that received maternal exposure to PCP. The present study demonstrates that chronic exposure to environmental levels of PCP alters plasma thyroid hormone levels, as well as the expression of genes associated with thyroid hormone signaling and metabolism in the HPT axis and liver, resulting in abnormal zebrafish development.

WE127

Effects of perfluorinated compounds on mitochondrial functions in early life stage of zebrafish (Danio rerio).

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Perfluoroalkyl aicds (PFAAs) are well known surfactants that are widely used in industrial and consumer applications, and are persistent in the environment and biota. Many studies have elucidated their toxicological effects on various targets, including the liver, nervous and endocrine systems, and fetal development. This wide range of toxicological effects may be brought about from damages in the mitochondria, as well as mitochondria-mediated oxidative stress. In this study, we exposed zebrafish embryos to perfluorooctane sulfonate (PFOS), perfluorooctanoic acid (PFOA), and perfluorononanoic acid (PFNA) to investigate potential adverse effects on mitochondrial functions. Zebrafish embryos were exposed to five different concentrations (0, 250, 500, 1000, and 2000 μg/L) of PFOS, PFOA, and PFNA, respectively. Embryos were randomly collected from adult zebrafish and exposed to chemicals from 2hpf to 120hpf. Mortality, hatchability, and malformation rate were observed. At 120hpf, zebrafish larvae were collected and their gene transcriptions were analyzed. Total of 8 genes involved in electron transport chain (*cytb, atp5a1*, *coxI*, and *coxIV*) and oxidative stress (*gpx1, gstπ, hmox1,* and *sod2*) were examined. There were no changes in gene expressions of larvae exposed to PFOS or PFOA. However, altered expressions were observed for PFNA exposure groups. PFNA, at 1000 μg/L, significantly upregulated transcript levels of mitochondrial genes *cytb* and *atp5a1*, and of oxidative stress response genes *sod2* and *gstπ*. These initial results draws attention to potential effects low concentrations of PFNA have on various mitochondrial functions. Assessment of damages in the mitochondrial membrane and mitochondrial DNA are under way. This work was supported by NRF 900-20150033, Republic of Korea.

WE128

Effects of neuroactive pharmaceuticals on gene expression profiles in zebrafish embryos and larvae

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Neuroactive and neurotoxic contaminants present growing concern to fish, both at individual and population levels. Neuroactive pharmaceuticals in particular can modulate molecular neurological pathways and consequently interfere with population-relevant behavior at low concentrations. Ecotoxicological investigations of neuromodulation and neurotoxicity can benefit from molecular endpoints in fish, such as expression profiles of representative genes. In this study, we investigated the expression of a set of genes in zebrafish *Danio rerio* early life stages after static exposure to neuroactive pharmaceuticals. Zebrafish embryos and larvae up to 5 days post fertilization (dpf) were exposed to venlafaxine (serotonin norepinephrine reuptake inhibitor antidepressant), carbamazepine (voltage-gated sodium channel inhibitor and GABA receptor agonist anticonvulsant) and oxazepam (benzodiazepine derivative anxiolytic) at concentrations in the μg/L range (1 nM - 10 μM). These compounds are relevant aquatic contaminants, and selected concentration ranges are representative of environmental situations. Embryos and larvae were exposed for short (during 24 h) and prolonged (starting at day 0) exposure periods, simulating acute and chronic exposure scenarios. Afterwards, there was the evaluation of gene expression in embryonic (2 to 3 dpf) and larval (5 dpf) stages. Gene expression was quantified in pooled samples (n=24 fish) using Sybr Green based quantitative real-time PCR (qPCR). Target genes consisted of two set of genes, proposed as (i) markers of exposure to neuroactive compounds (e.g. *cfos*, *hspb11*, *fkbp5*) and (ii) markers of neuromodulation (e.g. *bdnf*, *neurog1*, *neuroD1*). Reference genes were *bactin*, *elfa* and *18s*. Exposure concentrations were measured at the beginning and at the end of exposure by LC-MS/MS. Results of this study are discussed regarding their ecotoxicological implications and the relevance for the annotation of adverse outcomes pathways. In addition, molecular endpoints are suggested for application in bioassays or as biomarkers in future ecotoxicological studies.

WE129

Development of an -omics based detection tool to discriminate between endocrine-mediated activity and systemic toxicity of substances.

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A mechanistic identification of the events preceding changes in vitellogenin expression is crucial to differentiate systemic toxicity from endocrine modes-of-action (MoA). An adverse outcome pathway (AOP) framework for endocrine MoAs shows that reduced vitellogenin concentrations lead to reduced fecundity and consequently, to a declining trajectory at the population level. Substances with other MoAs, exerting systemic toxicity, might also result in reduced vitellogenin, and thus lead to an identical adverse outcome (AO), although the molecular initiating event (MIE) is different. An improvement in determination tools is thus needed to ensure a reliable discrimination of endocrine disruptors from chemicals substances with other MoAs in order to avoid regulatory action and a further elongated higher-tier testing. The main aim of this study is to develop a method to discriminate between altered vitellogenin expressions as a result of systemic toxicity and endocrine disruption. In the present study, we apply iTRAQ proteomic labeling techniques and qPCR assays to quantify and validate genomic expression changes in zebrafish by means of quantitative analysis of respective proteins and peptides. Gonad samples and samples of other tissues treated with endocrine disrupting substance in comparison to the control samples are analysed by mass spectrometry-based approaches to identify regulated pathway and modifications of protein expression. The observed changes in endocrine-mediated MoAs will be compared with the MoAs arising from samples treated with substance leading to systemic toxicity. These improved approaches will allow identification and validation of specific MoAs and discrimination between different MoAs, which could not be distinguished by the standard approach due to an identical adverse outcome. QPCR analysis will be performed in order to determine if changes in mRNA expression are indicative for the triggered proteomic response. Candidate genes for this discrimination tool are *igf1, star and lss* genes related to steroid biosynthesis for determination of endocrine disruption or *lfabp10a, CYP2E1 and GSH* genes regulated due to systemic toxicity. The obtained results will provide a detailed understanding of a mechanistically-based alternative approach for hazard assessment.

WE130
Gene transcription profiles during the first 32 days of zebrafish development: thyroid, steroid, digestive and biotransformation systems
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The zebrafish has become an important model for ecotoxicity testing. Especially the zebrafish embryo is increasingly used for the development of alternative tests since early life stages of fish are not considered laboratory animals according to EU legislation. In addition to observing apical endpoints such as mortality, growth and reproduction, the focus on mechanistically understanding toxicity is increasing, e.g. in the context of AOP development. This demand is also increasingly supported by regulatory bodies. To better understand toxicological processes in early life stages it is essential to describe the reference state of healthy organisms. In this study we sampled zebrafish at 25 time points during development: 1.5; 6; 16; 24; 36; 48; 60; 72; 84; 96 and 120 hpf (hours post fertilization), and every two days from 5 until 32 dpf (days post fertilization). We analysed transcript levels of ± 40 genes involved in the thyroid, steroid, digestive and biotransformation systems using QPCR. While it is well known that these systems play a key role in both developmental and toxicological processes, the timing of activation of their specific components is poorly understood. For example, the biotransformation capacity of zebrafish embryos still is a topic of debate, and effects of endocrine disruptors on development have been demonstrated – however without properly described mechanisms. For the steroid system we studied the enzymes involved in steroid hormone biosynthesis, as well as the steroid receptors. With respect to the thyroid system, we included key regulators of the hypothalamic-pituitary-thyroid axis, thyroid hormone synthesizing and activating machinery, as well as thyroid receptors. We analysed the exact timing of activation of different digestive enzymes (carbohydrates, lipids and proteins) which is especially important for understanding the transition to free-feeding larvae. For the biotransformation system, we studied Phase I (Cytochrome P450) but also Phase II metabolizing enzymes and drug transporters. In addition to providing information on gene activation, our data shed light on potential maternal transfer of specific mRNAs. This library of zebrafish gene transcription profiles is intended to function as a reference both for fundamental developmental studies, as well as for toxicological studies.

WE131
Using Multi-Omics Measurements to Distinguish Contaminant Responses in a Zebrafish Model
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Environmental monitoring of chemical effects is challenging due to the ever increasing number of chemicals and the complexity of environmental samples. Conventional toxicity testing using whole animal organisms provides critical information on biological responses; however, morphological parameters such as growth and survival may not be sensitive enough to detect adverse effects of sublethal exposures, a scenario which is more ecologically relevant. In comparison, the ability to detect changes at the RNAs, proteins, and/or metabolite levels with techniques such as transcriptomics and metabolomics can generate high-throughput and sensitive early indicators of potential sublethal effects. Recently, zebrafish (*Danio* rerio) embryo has gained considerable interests through OECD and REACH initiatives as an alternative and equivalent toxicity testing version to the adult stage. As early developmental stages are critical and sensitive to environmental perturbations, the combination of zebrafish larvae and multi-omics technologies can be a powerful tool in characterizing early indicators of chemical insults. In this study we utilized a combined targeted metabolomics and transcriptomic approach to evaluate contaminant toxicity in a zebrafish larvae model. Zebrafish larvae (96 hpf) were exposed to a wide classes of contaminants including pharmaceutical and personal care products and heavy metals at environmentally relevant concentrations for a 24h period. Quantitative real-time PCR were performed on over 40 target genes covering a wide range of biological systems to assess transcriptomic effects. At the same time, over 217 metabolites, including acylcarnitines, amino acids, glycerophospholipids, ∑hexose, sphingolipids, biogenic amines, fatty acids, and bile acids, were also quantitatively measured by LC-MS/MS and FI-MS/MS to determine the effects on the metabolome. The unique molecular signature response may be used to inform the types of toxicants present in the environment. Furthermore, this integrated OMICS approach can better inform the chemical modes-of-actions and allows a systems biology approach in identifying key trajectory that may lead to adverse health outcomes for exposed animals.

WE132
Toxic effects of sediments contaminated with metals in the zebrafish Danio rerio
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Heavy metals have as their main source of anthropogenic activities being the sediments one of main reservoirs of these elements, acting as sources of pollution by providing metal to the water column, which constitutes a threat to aquatic biota. Because there are few studies of the effect of sediments contaminated in fish, the aim of this study was to evaluate the toxic effect of sediments contaminated with Cd, Cu and its metals mixture on zebrafish. Bioassays were performed. The organisms were exposed to 3 sediment samples: 2 samples spiked with metals Cd (5 mg L^{-1}) and Cu (2.5 mg L^{-1}) and the third with the mixture of the two metals, for 20 days, plus a control group. At the end of the exposure period 5 organisms were taken at random from each experimental group, Its weight was evaluated and the following biomarkers: lipid peroxidation (MDA levels), and frequency of micronuclei. The results show that the rates of increase in weight of the organisms exposed to contaminated sediments, had negative values, indicating that fish lost biomass. Fish exposed to sediments contaminated with Cadmium and the mixture of metals showed higher levels of lipid peroxidation, (43.9 and 37.1 nM MDA g^{-1} respectively). The highest frequency of micronuclei (0.075%) was observed in organisms exposed to the mixture of metals. The results of this study indicate that sediments contaminated with metals cause adverse effects on organisms living in the water column.

WE133
Reactive oxygen species (ROS) quantification as an endpoint in mixture toxicity analysis with the zebrafish embryo (Danio rerio)
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Reactive oxygen species (ROS) serve as signaling molecules and are also a general side product of metabolism. The concentrations of ROS are counterbalanced by a group of antioxidant molecules and enzymes as increased concentrations will damage proteins, DNA and lipids and finally could lead to death of organisms due to oxidative stress. The embryos of the zebrafish are now an established model organism in ecotoxicology serving both as a tool for mode of action analysis and as a fish test alternative for regulatory and monitoring purposes. Some literature exists which already use the embryos or larvae of the zebrafish in immunological research. In the following work the existing published protocols were adapted to the use of the zebrafish embryo in ecotoxicology testing as described by the OECD 236 guideline. The different developmental stages of the embryos and eleutheroembryos from 2 to 96 hours post fertilisation (hpf) were analysed without and with ROS producing substances by the use of the fluorescent dye 2,7- dichlorodihydrofluorescein diacetate. The organism might show age and substance dependent different sensitivities and reactions to oxidative stressors. Substances known for their ROS generating potential served as positive controls (e.g. hydrogen peroxide, tert-butyl hydroperoxide (TBHP), rotenone and CdCl2) to evaluate sensitive time windows for exposure. The increased fluorescence due to oxidative stress was analysed on whole organisms in kinetic mode over a duration up to 6 hours in 96 well micro titer plates and with a fluorescence photometer. Depending on age and substance an increase in fluorescence could be observed within min (hydrogen peroxide) or only after many hours (TBHP). The reactions (in terms of relative fluorescence unit increase) ranged between 70 and 300 % of control embryos and the test proved to be suitable for the analysis of multiple samples in parallel within a maximum of 6 hours. Results will be presented also in the view of usage of the ROS-test for mode of action analysis of similar and dissimilar acting substances in mixtures of environmental contaminants.

WE134
Effects of Microcystis aeruginosa and the toxin microcystin-LR on target gene expression profiles and histopathological changes in larval zebrafish Danio rerio
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Microcystis aeruginosa is a cyanbacterium that can produce the hepatotoxin microcystin-LR (MC-LR) and that is responsible for many harmful algal blooms (HABs) in freshwaters. Blooms of *M. aeruginosa* frequently occur in eutrophic surface waters, and the presence of the cyanobacteria and toxins can become an important problem for ecological and human health. We have found that toxicity

in the zebrafish *Danio rerio* includes effects that are independent of the toxin. Our objectives are to investigate changes in target gene expression profiles in zebrafish larvae in relation to concentration and duration of exposure to *M. aeruginosa* and MC-LR, and to investigate the histopathology changes that could happen to the larvae during the exposure. We have cultured a toxin-producing strain and a non-toxin-producing strain of *M. aeruginosa* to generate a supply of lyophilized cyanobacterial cells characterized for use in toxicity tests. Zebrafish larvae (age 72 h post fertilization) were exposed to lyophilized *M. aeruginosa* or MC-LR for 96h at sub-lethal concentrations (5, 25, 50, 100, 200, 400 μg MC-LR/L). Also, another set of zebrafish larvae were exposed to MC-LR or *M. aeruginosa* at concentrations of 5, 50 μg MC-LR/L, and the larvae were sampled after different exposure durations (4, 24, 48, 72, 96 h). Survival of larvae was recorded, histopathology conducted, and changes in expression of target genes were investigated by quantitative PCR (Q-PCR) for the following genes: catalase (*CAT*), superoxide dismutase1 (*SOD1*), glutathione peroxidase (*GPx*), glutathione-S-transferase (*GST*), protein phosphatase (*PPP1, PPP2A*), vitellogenin (*VTG*), and cytochrome P450 (*CYP1A*). We anticipate that these results will enhance understanding of the molecular responses and the histopathological changes in fish during the progression of exposure to a HAB caused by *M. aeruginosa*. **Key words:** *Microcystis aeruginosa*, microcystin-LR, Gene expression, Zebrafish.

WE135

Detection of cholinesterase inhibitors in water samples from Novi Sad (Serbia) using effect-directed analysis

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Organophosphates and carbamates are commonly used as insecticides in agriculture, residential landscaping, public recreation areas and in public health pest control programs. The main known mode of action is the inhibition of cholinesterases (ChE) and the subsequent increase of acetylcholine in the synaptic cleft. For many of these substances the structure and fate is well characterized and they are routinely monitored in order to detect their concentrations in the ecosystems. The presence of organophosphates and carbamates in the environment can cause several problems and distress in the aquatic life such as malformations, damage to the nervous system and reproduction problems. Despite the clear mode of action (MoA), many factors could influence the results of an *in vivo* test of environmental samples during the analysis of AChE activity: mixture effects, developmental delay, malformations and the presence of other compounds with different MoA (e.g. dioxin-like compounds) are only some of the factors that could influence the bioassay. The complex environmental mixture could mask the effect or lead to false positive results. However, effect directed analysis (EDA) can identify masking effects, unravel the presence and identity of primarily causative chemicals by combining chromatographic fractionation, chemical analysis and biotesting. In this study, water samples of the river Danube (Novi Sad, Republic of Serbia) were collected using large volume SPE device with a polymeric sorbent. Chemical analysis with LC/HRMS showed presence of organophosphates in the parent sample of untreated wastewater. The sample was subsequently fractionated using a HPLC with a reversed phase semi-preparative C18 column and 26 fractions were collected. Preliminary results are showing a clear inhibition of AChE (20% compared to negative control) in only one out of 26 fractions. This study is trying to elucidate the contribution of the use of ACHE enzyme activity inhibition of substance groups like organophosphates and carbamates in the frame of *in vivo* testing of environmental (mixture) samples and EDA studies with *Danio rerio* embryos.

WE136

Effect-directed analysis of atmospheric particulate matter

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Atmospheric particulate matter (PM) is a recognized risk factor for the global burden of disease in human populations and a widespread threat to all living organisms. PM is primarily emitted from combustion processes and comprises a significant fraction of organic compounds, such as polycyclic aromatic hydrocarbons (PAHs). If specific tools could be developed to promptly discriminate the toxic potential of PM, based on the mode of action of its constituents, rather than on PM mass and BaP concentrations which are currently used in air quality legislation, a more straightforward approach to air quality management could be accomplished. Therefore, our main objective was to investigate the toxicity of the organic fraction of atmospheric PM and provide a cost-effective approach that could be easily applied to diagnose potential toxicity of exposure to this fraction. To tackle this objective, a general strategy was adopted, in which a yeast-based assay for dioxin-like activity (AhR-RYA), and the zebrafish embryotoxicity assay (ZET assay) followed by molecular tools, were used to evaluate biological effects elicited PM1 (PM< 1 µm) organic extracts from the city center of Barcelona. This study also clarified whether the effects observed can be further assigned to primary or secondary PM organic components, and whether time differences (day vs. night, working days vs. weekends) translate into distinctive effects of PM constituents. The work developed lead to the major conclusion that the AhR-RYA and the ZET are valuable assays for effect-directed analysis of ambient PM, also allowing to study the mode of action of PM organic fraction. Integrated results from different surveys from diverse sampling points suggest that physiological effects are strongly dependent on the chemical composition, particle size distribution, and, ultimately, the emission sources of PM. On the contrary, total PM mass and BaP concentrations appeared as poor predictors of potentially adverse biological effects. Therefore, effect-directed air quality assessment would likely represent a major improvement on PM toxicity evaluations. The authors acknowledge the CTM project (CTM2014-51985-R), and the Portuguese Foundation for Science and Technology for the doctoral grant of S.R. Mesquita (SFRH/BD/80710/2011) funded by the Program POPH-QREN through the Portuguese Ministry of Education and Science and the European Social Fund, and support through project PEst-C/MAR/LA0015/2013.

WE137

Development of a refined zebrafish feeding trial

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Fish feeding trials are important for investigating foodborne ecotoxicological exposure as well as for evaluating food safety. Although a dietary biomagnification fish test exists, a standardized fish oral toxicity test is currently not available. Safety of food components, additives and contaminants is generally evaluated using rat feeding trials to assess (repro)toxicity. The development of a refined zebrafish feeding trial would advance existing testing strategies by reducing the cost, by replacing the use of mammals/birds by a lower vertebrate species, and by facilitating reproductive and multigenerational studies. When testing food, substitution of part of the food by the component under evaluation is often required, possibly interfering with nutritional requirements. Therefore, the extent of component substitution which can be processed by the zebrafish metabolism (maximum tolerable percentage) should be assessed prior to the component evaluation trial. Since we will conduct a feeding trial with GM maize later on in our study, we defined the maximum tolerable percentage of maize. First, we formulated experimental fish feeds based on the composition of commercial fish feeds. Maize was introduced in the feed by a stepwise substitution of a wheat component (25% of the whole feed). We compared 6 experimental feeds to three different commercial feeds in a one month feeding trial, during which we investigated the following endpoints: condition factor and growth, hepatosomatic and gonadosomatic index (HSI and GSI), reproduction, energy budget and feed digestibility. The growth of fish fed with 25% wheat or 25% maize slightly decreased. The HSI of males increased with increasing percentage of maize. Feed digestibility analysis showed a decrease in carbohydrate uptake when fish were fed with an increasing percentage of maize substitution. Based on these results, we selected a 15% maize substitution for conducting the GM maize feeding trial. This selection was based on two criteria: (1) the biological limits of the maize component in the zebrafish feed should be respected, (2) the amount of maize substitution should allow us to observe effects of GM maize if there are any. We suggest that our approach of determining component substitution rates before carrying out feeding trials could be a valuable asset when evaluating food safety, as well as for investigating dietary ecotoxicological exposure of fish.

WE138

Comparison of Different Feeding Diets to Improve the Performance of Higher-Tier Fish Tests

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For the testing of endocrine functions of substances, the demand for higher-tier/ multi-generation fish tests like the MEOGRT (OECD 240) constantly growths. Such tests usually investigate at least one complete generation cycle of the F1 generation. The first two to three weeks post hatch is the most sensitive and critical phase. Feeding during this period of time is crucial for the development of fry and therefore the successful performance of the tests. Since higher-tier fish tests are time consuming and complex, feeding of fish should be kept as simple as possible. Therefore, artificial foods like micro flakes are often the preferred foods of choice. However, our results showed that artificial foods were not sufficient for the sole feeding of fry. The Early Life Stage Test (ELS; OECD 210) is a 35-day-test which exactly covers the critical first life stages of fish and therefore is greatly suitable to investigate development of fry. In order to investigate the optimal diet for fry in our higher-tier fish tests, we performed several ELS tests according to OECD guideline 210 with the Zebra fish *Danio rerio* in a flow-through system. We compared different diets consisting of commercial micro flake and liquid fry food, hole egg suspension, different living brine shrimp species and decapsulated brine shrimp, rotifers and combinations of these. After 35 days survival, growth and weight were compared. The tests showed that Zebra fish fry was too small to feed on most artificial foods. Further treatment like grinding was necessary increasing time for preparation. Our results indicated a significant preference for natural diets like brine shrimp and rotifers with most survival rates higher than 90 %. The poster will compare the results of the different diets and give hints on which diet may be preferred in higher-tier fish tests. Furthermore, the existing validity criteria of standardized higher-tier fish tests will critically be reviewed in comparison to our findings.

WE139

Xenobiotic metabolism in alligator gar (Atractosteus spatula): A comparison with rainbow trout (Oncorhynchus mykiss)

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Metabolic stability determines the extent to which xenobiotics are biodegraded. One of our interests is pharmaceutical compounds, which are chemicals of emerging concern in aquatic environments. Information on the chemical metabolism can be used to determine bioaccumulation potential or BCF (Bioconcentration factor) that is used for regulatory purposes in REACH (Registration, Evaluation, Authorization and Restriction of Chemicals). Under this initiative, chemicals are regulated and must be tested for PBT (Persistency, Bioaccumulation and Toxicity). The trout *in vitro* metabolism assay is a new *in vitro* methodology to test for bioaccumulation in the PBT assessment. This assay is undergoing validation through the OECD (OECD Project 3.13) and is gaining acceptance among regulatory agencies. The *in vitro* trout metabolism assay decreases the number of animals used for testing, testing costs and time required to obtain results. Despite of the advances in this and other methodologies, we must evaluate *in vitro* metabolism assays across a variety of species to better understand interspecies variation and differences in how species function under different or environmentally relevant conditions such as dissolved oxygen concentrations and temperatures. The objective of this research was to compare *in vitro* metabolism in the liver S9 fraction of alligator gar to rainbow trout as our reference species. Alligator gar is a unique ancient fish that provides a valuable contrasting model to rainbow trout. Alligator gar live at higher temperatures and lower dissolved oxygen levels than rainbow trout. Differences may be found in how these two species do or do not metabolize xenobiotics. Laboratory or hatchery raised alligator gar and rainbow trout were maintained at 23?C and 13?C, respectively. Fish were acclimated to laboratory conditions for at least one-month prior to sacrifice. Fish were euthanized and livers from alligator gar and rainbow trout were perfused with a saline buffer (HBSS) at pH 7.8. After each perfusion, liver S9 fractions were prepared following protocols established for rainbow trout (Johanning et al., 2012). Phase I and II enzyme activities for both species were compared. Thus far, EROD and GST activities appear to be comparable. Experiments are ongoing to further compare metabolism by these enzymes and metabolism of pharmaceuticals to explore evolutionary strategies and adaptations at the physiological level.

WE140

Endocrine disruptor risk evaluation in vivo with transgenic zebrafish larvae indicating glucocorticoid signaling activity

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Glucocorticoids (GCs) are steroid hormones with important functions in metabolism and stress response. Synthetic GCs have immuno-suppressive, anti-inflammatory and anti-allergic properties and are widely prescribed for the treatment of diseases such as asthma and rheumatoid arthritis. These drugs are released into the environment due to incomplete removal by waste water treatment plants, and remarkably high concentrations were detected in receiving rivers. Acting as endocrine disruptors, these and other compounds may interfere with the hormonal system of wildlife, calling for *in vivo* tests measuring GC activity. Currently available test systems are centered on *in vitro* reporter gene bioassays with transgenic cell cultures. In contrast, bioassays with zebrafish larvae enable risk assessment in the whole, living organism. Our ***G**lucocorticoid* **R**esponsive **I**n vivo **Z**ebrafish **L**uciferase activit**y** (**GRIZLY**) assay allows us to investigate activation or inhibition of GC signaling *in vivo*, taking into account effects such as metabolization of compounds or organ specific effects.The assay is based on a transgenic line (*GRE:Luc*) carrying a luciferase reporter gene under control of four GC response elements (4xGRE). Comparison with a zebrafish cell line carrying the same reporter construct helps to pinpoint indirect or metabolism-related *in vivo* effects of a sample. The **GRIZLY** assay identified known activators of GC signaling, including the environmental contaminants dexamethasone, prednisolone and prednisone, in a chemical library of pharmaceutical compounds. It also detected pregnenolone, which stimulates endogenous cortisol production and, therefore, was active only in the larval assay. When screening for inhibition of GC signaling, we again found compounds active *in vitro* and *in vivo* as well as numerous substances only active *in vivo*. Importantly, the test can equally be modified to specifically target compounds interfering with endogenous GC biosynthesis. Strikingly, several drugs so far not implicated in interfering with GC signaling were identified by the larval assays. Deeper investigations of their underlying modes of action are in process. These results point out that interference with GC signaling and biosynthesis needs more attention in environmental risk assessment of compounds, and that *in vivo* testing is indispensable for this. Our test system covers these needs, allowing rapid whole-organism testing with high sensitivity and specificity.

WE141

Linking gene induction to physiological effects using transgenic medaka

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Gonadal development in medaka (*Oryzias latipes*) is dependent on the synergy between oestrogens and androgens. Any disruption in steroid hormone levels can cause the induction of ovo-testis, a clear indicator of sex reversed germ cells. To determine the sensitive windows for hormonally induced sex reversal in medaka, we developed a novel 42sp50-GFP_ChgH-GFP transgenic medaka line. This line allows the identification of ovo-testis by fluorescence (GFP) present in developing oocytes and in parallel allows quantification of oestrogen axis activity via GFP in the liver. Germinal transgenesis resulted in a stable line exhibiting strong GFP signal in the ovaries and liver in response to oestrogens. To identify the sensitive developmental period to exogenous agents, fry were exposed to 24 hour pulses of oestradiol (E2; 500μg/L), dihydrotestosterone (DHT; 2.5μg/L or 500μg/L) or flutamide (5mg/L) at various time points between day post fertilisation (dpf) 1 and 12. Evaluation of phenotype followed by genotyping at dpf 16, revealed sensitivity to E2 between dpf 1-5 and two periods of increased susceptibility to DHT or flutamide between dpf 0-1 and dpf 5-8. No phenotypic sex reversal was detected after the exposure to DHT or E2 on dpf 11 or dpf 12 respectively. We show the relative sensitivity of early developmental stages to the induction of ovo-testis in XY and XX medaka and that our newly developed 42sp50-GFP_ChgH-GFP medaka line is a suitable model for studying this phenomenon.

WE142

Synthetic progestins disrupt the glial cell specific-brain aromatase expression in the developing brain of fish

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Few studies have addressed the biological effects of natural and synthetic ligands of the nuclear progesterone receptor (nPR) on fish development and reproduction, resulting in a significant lack of data to assess the hazard and risk posed by these compounds on aquatic organisms. Yet progesterone plays key roles in many vertebrates and recent data showed that some progestins are present in the aquatic environment at concentrations that can impair reproduction. In light of this context, we studied the effects of progesterone and a panel of synthetic nPR ligands on the estrogen-receptor (ER)-signaling pathway by investigating the expression of the zebrafish brain aromatase *cyp19a1b* gene using zebrafish-specific mechanism-based *in vitro* and *in vivo* assays. Twenty-four nPR ligands were screened on transgenic *cyp19a1b*-GFP zebrafish embryos to assess their potential estrogenic effect. Progesterone and its isomer dydrogesterone as well as drospirenone and all the progesterone-derived progestins had no effect on GFP expression. However, all progestins derived from 19-nortesterone induced the *cyp19a1b* expression in an ER- and concentration-dependent manner with EC_{50} ranging from the low nM to the μM range. These results show that progestins are estrogenic to fish and that early-life exposure of fish to 19-nortestosterone disrupts the ER-signaling pathway within the developing brain. The 19-nortestosterone derived progestins levonorgestrel and norethindrone were further tested in U251-MG cells transfected with any of the three zebrafish ER subtypes zfERα, zfERβ1, or zfERβ2. Progesterone had no effect on luciferase activity with either of the zfER subtypes. Norethindrone and levonorgestrel both induced luciferase activity that was blocked by co-exposing the cells with ICI 182,780. An ER competition assay showed that both progestins were unable to bind to and activate ER except at very high concentrations (10^{-5}M), suggesting they require metabolic activation prior to elicit estrogenic activity. This study highlights the relevance of using zebrafish-specific screening assays to characterize endocrine disrupting properties of emerging contaminants and demonstrates that 19-nortestosterone derived progestins are pro-estrogenic compounds targeting radial glial cells inducing *cyp19a1b* expression in the developing brain of fish. Given that ER signaling is one of the very first to emerge during early-life stage development, the consequences should be further investigated.

WE143

Nonylphenol impairs development of the olfactory bulb by altering projection networks of estrogen responsive cells in the forebrain

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We have established an estrogen biosensor transgenic zebrafish (ERE-TG fish) that detects tissue responses to estrogenic chemicals via expression of fluorescent reporter proteins.. Using the ERE-TG fish, we have observed that specific subsets of cells in the forebrain are responsive to estrogens, however, the role(s) of estrogen signalling in development and function(s) of these cells are not known. We observed two types of estrogen responsive cells in the forebrain cells from the early onset of neurogenesis in the ERE-TG embryo, one localised in olfactory bulb with complex projection networks and another in telencephalon showing a characteristic polar morphology with a radial process. These estrogen responsive cells were not immunoreactive for neuronal markers (acetylated tubulin and HuC), but expressed a neural progenitor marker Sox2, suggesting specific roles of estrogen signalling in the neural progenitor development/ function(s). We investigated the spatiotemporal relationships between olfactory sensory neurons and estrogen responsive olfactory progenitor cells, applying fluorescent *in situ* hybridization for olfactory bulb neuron specific markers (lhx2 and tbx21) and immunohistochemistry against the ERE reporter (mCherry). Estrogen responsive olfactory cells were localised at spatially distinct regions from those of lhx2 or tbx21 positive cells but their projections innervated directionally towards lhx2 and tbx21 expressing cells, suggesting the estrogen responsive olfactory bulb cells may play role(s) the development/function(s) of lhx2 and tbx21 positive olfactory sensory neurons. Interestingly, exposure to an endocrine disrupting chemical (nonylphenol, NP) markedly inhibited the establishment of projection networks of the estrogen responsive olfactory cells, which showed shorter projections and ectopic distributions of the cell bodies. NP did not alter the spatiotemporal expression of lhx2 or tbx21 but impaired the innervation of estrogen responding olfactory cells to Lhx2 and tbx21 positive cells. NP also reduced significantly the size of olfactory bulb/forebrain. Taken together, our findings indicate estrogen responsive cells in the forebrain possess fundamental roles in olfactory bulb developmentand the environmental oestrogen NP may affect brain development during embryogenesis by impairing development of estrogen responsive olfactory cells and their spatiotemporal networks.

Wildlife ecotoxicology: from food chain exposure to population effects (P)

WE144

Effects of Persistent Organic Pollutants on Vitamin A Levels in Faroese Pilot Whales

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Long-finned pilot whales (*Globicephala melas*) are highly exposed to persistent organic pollutants (POPs), such as those classified as organohalogen contaminants (OHCs). POPs are known to interfere with the regulation and homeostasis of retinoids (vitamin A and its metabolites). Retinoids are lipophilic molecules essential for vision, growth, reproduction, immune function and cellular division and differentiation in mammals. Disruption of retinoid homeostasis can thus lead to impairment of these functions in mammals. OHCs and their metabolites can interfere with retinoids by different mechanisms including the disruption of vitamin A metabolism. The CYP26 enzyme is involved in the metabolism of retinoids by catalysing the oxidation of retinoic acid to polar metabolites. Effects of POP exposure on vitamin A in pilot whales were presently studied by analysing correlative relationships between the concentrations of selected OHCs (PCBs, OCPs and PBDEs) and retinoids in plasma and liver and *cyp*26 mRNA expressions in liver of the pilot whales. The results showed that *cyp*26a1 mRNA expression, but not *cyp*26b1 expression, was inversely correlated to all analysed POPs (r_s0.543, $p< 0.05$). The higher retinol levels with increasing POP concentrations in plasma could indicate increased mobilisation of retinol, although the effect was not detected in the liver stores.

WE145

Contamination of farmland bird's eggs by present and past plant protection products - the grey partridge as a case study

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Farmland birds are in decline in Europe. The contamination of their eggs by currently used plant protection products (PPPs) is poorly documented despite a potential to adversely impact their breeding performance. In this context, 139 eggs of 52 grey partridge *Perdix perdix* clutches were analysed using GC/MS-MS and LC/MS-MS screenings measuring ca. 500 compounds at a LoQ of 0.01 mg/kg. A total of 15 different compounds were detected in 24 clutches. Nine of them are currently used by farmers as fungicides (Difenoconazole, Tebuconazole, Cyproconazole, Fenpropidin, and Prochloraz), insecticides (lambda-Cyhalothrine and Thiamethoxam/Clothianidine), and herbicides (Bromoxynil and Diflufenican). Mixtures were less detected than expected by potential exposure. We could associate a contamination and a probable exposition (field treatment) in several cases. Some older PPPs were also detected (Fipronil (+sulfone), HCH (a,b,d), Diphenylamine, Heptachlore (+epoxyde), DDT (Sisomers)), as well as PCBs. Concentrations ranged between < 0.01 and 0.05 mg/kg, but reached 0.067, 0.11, and 0.34 mg/kg in three cases. Our results testify a contamination of females and/or their eggs to PPPs in operational conditions, as well as to organochlorine pollutants or their residues, banned inFrancesince years, even decades, that persistently contaminate the environment. We discuss the possible routes of exposure, the probability to detect a contamination in the eggs, and the prevalence of such a contamination by means of multicriteria analysis. Unfortunately, although the wealth of information collected and analysed, it remains difficult to conclude about effects on egg / embryo characteristics yet, and additional investigations are needed. Lastly, we share our experience from our case study to document unintentional effects of PPPs on the breeding success of wild birds in the real world. This work was supported by the research programme *Pesticides* funded by the French Ministries in charge of Ecology and Agriculture with a financial support from ONEMA. Funds issue from the fees on diffuse pollution.

WE146

Exposure of Red Kite nestlings to chemicals and consequences on health

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The Red kite is a European endemic raptor listed as "Near-Threatened" by the IUCN. Poisoning by pesticides is considered one of the major threats for kites and they can be exposed to metal(loid)s, and/or POPs. Within the framework of the French National Action Plan, some breeding populations are monitored, several tens to hundreds of chicks being ringed each year since 2006. This offers an opportunity for blood sampling in order to assess exposure to chemicals. Here, we present the results from a monitoring done on the Red kite in France during springtime 2013 and 2015. Blood was sampled from 121 chicks in 2013, metal(loid)s, rodenticides, PAHs and OCls were measured and related to body condition and breeding success. In 2015, analyses on different health indicators are currently in progress on 50 blood samples and results will be presented at the congress.

WE147

Emerging chemicals in birds of prey from Spain and Norway: exposure and effects

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In 2014, the international research project NewRaptor was launched (funded by the Norwegian Research Council and the Norwegian University of Science and Technology). In this project, we investigate the exposure and effects of emerging chemicals in birds of prey from three regions (Murcia, southern Spain; Trøndelag, mid Norway; Troms, northern Norway). The goshawk (*Accipiter gentilis*) was studied in all three regions, while the marine white-tailed eagle (*Haliaeetus albicilla*) only in the two Norwegian locations. Both blood and body feathers were obtained from the chicks in the nest when they were 3-7 weeks old. The chemicals of interest include novel brominated flame retardants (nBFRs), organophosphate flame retardants (OPFRs) and per- and polyfluoroalkyl substances (PFASs). In addition, metals and legacy chemicals, i.e. persistent organic pollutants (POPs), were analysed as well. The objectives of the NewRaptor project are to evaluate the exposure of these two bird species to both emerging and legacy compounds in relation to habitat and dietary preferences (using stable isotope analysis), differences in latitude, and to evaluate the interactive effects of the pollutant mixtures on different biochemical, immunological and endocrine parameters. The first results of the sampling campaign in 2015 indicate that the PFAS exposure in goshawks from Murcia are lower than in Norwegian chicks, with up to 10 times higher values in Norway: e.g. linear PFOS < LOD-3.1 ng/ml in Murcia; 4.9-30 ng/ml in Trøndelag; 0.72-24 ng/ml in Troms). Regarding the white-tailed eagle, linear and branched PFOS were the most prominent PFAS (linPFOS: 6.0-32 ng/ml in Trøndelag;

WE148
Age-dependent changes in oxidative stress biomarkers and essential elements in blood of red deer
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Changes in the concentration of the essential elements in the blood of animals throughout their life may be indicative of periods of greatest vulnerability to deficiencies and associated diseases. Here we have studied the changes in antioxidant biomarkers and essential elements in the blood of females of farmed red deer along their life. These changes are discussed in relationship with the incidence of enzootic ataxia, white muscle disease and reproductive failure in farmed deer. Blood samples of female red deer (n=132) were analysed to determine the concentrations of essential elements with roles as cofactors in antioxidant enzymes (GPx and SOD). Moreover the activity of there antioxidant enzymes and antioxidant vitamins (retinol and α-tocopherol) was measured in the same blood samples. In addition, samples of plants, soil and water from the same study area were analysed to identify possible deficiencies and interactions of trace elements in the diet of the studied deer. Levels of Zn and Mn in blood decreased with age, whereas Se and Cu levels showed an increase during the first year of age and then stabilized at slightly lower levels the following years. Farmed deer showed deficiency levels of Cu during most of their life and, at a lesser extent, were also Se-deficient. The activity of GPX increased along the life of deer, while a decreasing trend was found for SOD. In the case of vitamins, retinol increased during the first year of age and tocopherol peaked at 3-4 years of age. Positive correlations were observed between the blood levels of Se and GPx activity and between levels of Zn/Cu/Mn and SOD activity. These results indicate that trace element deficiencies described above (especially Cu) may have an effect on the antioxidant capacity (especially SOD activity) of the animals at adult stages, when reproduction effort may increase antioxidant requirements. Cu deficiency in farmed deer could be related to low levels of Cu in soil of the study area or to elevated levels or sulphates in water that could lead to the formation of non-bioavailable forms of Cu in the digestive tract of deer. In summary, the use of enzymatic and non-enzymatic antioxidants as effect biomarkers of the exposure to pollutants should be always considered taking into account the age of the animals and the levels of essential elements acting as antioxidant cofactors.

An in silico modelling perspective to advance hazard assessment of aquatic ecotoxicology (P)

WE149
Linking algal growth inhibition to chemical activity: A tool for identifying excess toxicity
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Aquatic toxicity data are most often expressed on a concentration basis (e.g., EC50 in mg/L). Whereas the data are useful within regulatory risk assessment, a given EC50 value offers no direct information on whether the compound exerts baseline toxicity or excess toxicity. Chemical activity (a) is a medium-independent exposure parameter that quantifies the energetic level, and not the concentration, of an organic compound relative to its energetic level in pure liquid [0-1, unitless]. Several studies have linked toxicity to chemical activity, and baseline toxicity (narcosis) for neutral hydrophobic organic compounds has been shown to initiate in the narrow chemical activity range of 0.01 to 0.1. These findings suggest EC50 values for baseline toxicity to be at or above 1% of liquid saturation, and EC50 values well below this limit do thus indicate excess toxicity. In the present study, published toxicity data were expressed on a chemical activity basis in order to identify excess toxicity. More specifically, algal growth inhibition was linked to chemical activity using two approaches: (1) algal toxicity data were plotted relative to a regression for the (sub-cooled) liquid solubility, which served as a visual reference for chemical activity of unity and (2) ratios of algal EC50 values and (sub-cooled) liquid solubilities were determined, since the this ratio essentially equals the effective chemical activity (Ea50). In both approaches, compounds exerting toxicity well below the limit of 1% of liquid saturation (a=0.01) were identified as compounds exerting excess toxicity and investigated further. Also, the two approaches were compared on both a methodological and scientific level. In the present study, toxicity data came from several comprehensive and carefully conducted algal growth inhibition experiments covering a wide range of solid and liquid organic compounds, covering several expected modes of action and also several algal species. This presentation shows how the chemical activity concept can be used to compare and combine toxicity data across compounds and species in order to characterise toxicity – and further how the concept can be used in environmental risk assessment.

WE150
High-accuracy water solubility determination using log KOW from different methods
P. Bicherel, P. Thomas, KREATiS
Work was carried out to determine the relative accuracy of several experimental methods used to determine log K_{OW} values: HPLC (OECD 117); shake flask (OECD 107) and slow-stir (OECD 123). In order to do this log K_{OW} values were plotted against water solubility values. It was found that the method for log K_{OW} determination directly influenced the relationships found between log K_{OW} and water solubility. Water solubility is a parameter which is typically measured with a shake flask method (OECD 105) or improved using a slow-stirring method (derived from OECD 123 for log K_{OW} determination) when water solubility is low. When log K_{OW} data for a group of compounds (e.g. oxygenated substances excluding alcohols and acids) are divided between shake flask and slow-stir methods and compared to results from the HPLC method, goodness-of-fit of correlation are significantly different. Water solubility values correlate better with log K_{OW} values determined using shake flask or slow-stirring methods. However HPLC method overestimates log K_{OW} with an average error of 0.43 log units. HPLC cannot be used as a reliable method to measure log K_{OW} with accuracy and this is also the reason why HPLC data should be excluded as a data source during development of High-Accuracy QSAR models.

WE151
Analysis of sorption of non-ionic and anionic surfactants to different stationary phases
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Anionic and non-ionic surfactants are high-production volume chemicals that are found in many consumer products and consequently also in the environment as organic contaminants. Octanol-water partition coefficients (K_{ow}) are often used in QSAR studies as a measure of hydrophobicity to predict the environmental fate and exposure of neutral organic compounds. However, K_{ow} is not meaningful for surface-active compounds because sorption to environmental matrices is not only driven by their hydrophobicity, but also by specific interactions with the sorbent (hydrogen-bonding and electrostatic interactions). Furthermore, experimental K_{ow} values of both non-ionic and anionic surfactants are difficult to determine because of their surface active properties and ability to emulsify the octanol-water system. Alternative approaches to quantify and predict the environmental behaviour of surfactants are therefore required. Our research is focused on developing and testing new approaches and parameters that can be applied in models to predict sorption and bioaccumulation of different surfactant classes. To this end we studied the interactions between surfactants and different stationary phases (C_{18}, Hydrophilic Interaction liquid chromatography (HILIC), and ion exchange) with a liquid chromatographic method. Retention times of surfactants were measured and capacity factors were extrapolated to 100% water (k'_0). The affinity of surfactants for the C_{18} phase increased with carbon chain length for both anionic and non-ionic surfactants. Fragment values (log D*k*) were calculated by multiple regression analysis of log k'_0 values for all surfactants. These values showed that hydrocarbon and fluorocarbon moieties contributed most to hydrophobicity while anionic head groups such as carboxylate, sulfonate, and sulfate groups contributed much less to hydrophobicity. All fragment values determined for the C_{18} phase could be interpreted through identification of interactions between surfactants and the aqueous phase (hydrogen-bond acceptors/donors, charged head groups) and are a good measure for the hydrophobicity of surfactants.Capacity factors for all stationary phases together may be applied as parameter in predictive models for sorption to specific environmental phases (e.g., membrane lipids or organic matter).

WE152
Reliability of BMD and ECx calculations required by Regulation EU 283/2013 for bird and mammal reproduction studies
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The ECx is a key endpoint for the long-term risk assessments of chemicals, including pesticides. Aquatic ecotoxicologists have since long argued in favor of using a defined point estimate (e.g. the EC_{10}) instead of the NOEC in longterm risk assessment. For avian and mammalian studies however, reliance has always been more on NOAELs as determined with statistical significance testing. Only recently EU regulation 283/2013 introduced the ECx in pesticide hazard identification and risk assessment, for all ecotoxicological areas, including birds and mammals. ECx endpoints are much better suited than NOAEL or LOAEL

also for combined assessments, e.g. in mixture toxicity evaluations, or for geometric mean calculations in case data are available from multiple studies or additional species testing. However, studies conducted, or evaluated, for bird and wild mammal longterm risk assessments are different from guideline studies in other ecotoxicological areas, with a comparatively high number of endpoints, but generated in test regimes with typically only three dose levels plus control. Therefore, ECx evaluation of such studies needs careful consideration. EU 283/2013 stresses the importance of reliable endpoints for risk assessment; this applies, of course, also to the calculation of ECx values. Currently, different approaches are used for calculation of ECx for bird and mammal studies, which are based on different principles and have a different historical background. Benchmark dose (BMD, equivalent to ECx) calculations are one of the newer approaches, developed for human toxicology and based on the precautionary principle. The key principle of BMDs is that different functions are compared and finally a conservative one is chosen to determine the BMD and its confidence intervals. In ecotoxicology, conventionally only a few (or even a single) dose-response functions are used and different methods are used to select the most adequate one. The selection of dose-response curves in ecotoxicology often follows other principles than the BMD approach. In the present study benefits and drawbacks of the calculation methods are compared and a new approach is proposed for calculation of robust and reliable ECx values. The proposed approach integrates the benefits of existing methods and provides endpoints that comply with the reliability criteria and is based on the OECD guidance for statistics in the analysis of ecotoxicity data.

WE153

A unique QSAR model to determine microorganism toxicity in activated sewage sludge

P. Thomas, J. Oses, P. Bicherel, KREATiS

Toxicity of microorganisms, usually following the Activated Sludge Respiration Inhibition Test (ASRIT) – OECD guideline 209 is a required endpoint in fulfilment of Annex VIII in REACH dossiers i.e. substances produced in quantities >10 TPA. Physicochemical properties (like adsorption to sludge or volatility) as well as the lack of obligation for analytical quantification makes it difficult to perform this test for some substances and interpretation can be ambiguous. In order to replace this kind of experimentation a High-Accuracy QSAR model has been developed. This work has been carried out as a part of the DAMIER project, a French funded project for the development and the use of High Accuracy QSAR models for REACH compliance. Therefore the model meets the five OECD principles (OECD, 2004) and has been validated to provide accurate EC50 values for non-polar narcotic substances. The algorithm is based on a simple linear relationship which links toxicity to activated sludge microorganisms to sub-cooled liquid solubility. It is based on the concept that chemical activity expressed through water solubility can explain aquatic toxicity as has previously been shown for fish, invertebrates and algae (Mackay *et al.*, 2009; Thomas *et al.*, 2015). This is the first model in ecotoxicity able to predict such an endpoint for regulation known to the authors.

Mechanistic effect modelling for risk assessment: state of the art, applications, use in a regulatory context and future directions (P)

WE154

A DEB analysis of responses to baseline toxicants in C. dubia and D. magna

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Mechanistic effects models are gaining interest in the scientific community and in regulatory settings. These models have the potential to facilitate species extrapolation, thereby decreasing the need for toxicity testing for risk assessment. They also provide an opportunity to quantify the complexities of multiple interacting stressors on environmental scenarios. Dynamic energy budget (DEB) theory represents a unifying framework for assessing the mechanisms that drive toxicant effects on life history traits. We report here on the progress of two case studies for an example chemical tested on two species of Cladocerans, *Ceriodaphnia dubia* and *Daphnia magna*. Over 70% of the ingredients in home and personal care products are considered baseline toxicants. We use phenol as a model baseline toxicant. A dynamic energy budget model was calibrated for each species, and differences in parameters and physiological mode of action are discussed. This work also explores how DEB based modelling can incorporate environmental factors such as food availability and temperature into risk assessment. In conclusion, the present study demonstrates the potential utility of DEB based models for species extrapolation and chemical risk assessment in an AOP framework.

WE155

Calculation of exposure-specific species sensitivity distributions by toxicokinetic-toxicodynamic modelling for the insecticide compounds imidacloprid, chlorpyrifos, and lambda-cyhalothrin

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Species sensitivity distributions (SSDs) are statistical distributions describing the variation among a set of species sensitivity towards a certain compound or mixture. An SSD can be made by fitting a statistical distribution through the results of acute or chronic toxicity tests for several species. SSDs can be used in environmental risk assessment of chemicals in two different ways, either for defining Environmental Quality Criteria (EQC), such as the often used HC5 concentration that is thought to be protective for 95% of all species in a community, or to estimate the fraction of potential affected species (PAF). In this work, we used toxicokinetic-toxicodynamic (TK/TD) models that were already parameterised for the insecticide compounds imidacloprid, chlorpyrifos, and lambda-cyhalothrin to generate model-based toxicity values for a series of time-variable exposure scenarios. Exposures contained different time-variable exposures, or chronic exposures over prolonged times, up to 100 days. SSDs were then generated based on these toxicity values, and could be compared with experiment-based SSD. The aim of this study is to assess how protective SSDs based on acute toxicity experiments are in the light of environmentally relevant time-variable exposure concentrations. Model-based HC5 and HC50 values were compared with respective values from experiment-based SSDs for chlorpyrifos, and hardly any differences could be identified, hence the method can be considered to work. Current results indicate that only small differences in HC5 values for the investigated compounds are visible for the different exposure profiles. This depends on the TKTD characteristics of the chemical, the type of TK/TD model used and the length and type of the time-variable exposure regimes. Further results will investigate also HC50 values which are supposed to show larger differences.

WE156

Application of Toxicokinetic-Toxicodynamic Modelling of Effects of Chlorpyrifos-Methyl on the Survival of Aquatic Macroinvertebrates for Environmental Risk Assessment

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In this study, toxicokinetic/toxicodynamic (TK/TD) models were calibrated, validated, and used for predictions for analysing effects of chlorpyrifos-methyl (CPF-M) on the survival of the mayfly, *Cloeon dipterum*, the crustacean, *Gammarus pulex*, and the dipteran, *Chironomus riparius*. Model calibration was performed based on data from laboratory toxicity studies conducted with the three species and CPF-M, by fitting the model parameters to the data and calculating their confidence limits. The parameterised models were validated with independent data on the toxicity of chlorpyrifos (CPF), because there were no appropriate datasets for testing available for CPF-M. The calibrated and as far as possible validated model parameters were used to predict effects of concentration time series selected from 133 FOCUS Surface Water Step 4 exposure scenarios on the survival of the three macroinvertebrate species. The ultimate objective was to check that if CPF-M is applied according to the proposed application scenarios, then exposure only leads to negligible levels of individual mortality for the three sensitive macroinvertebrate species in edge-of-field water bodies. For *C. dipterum*, the least sensitive of the three investigated species, deterministic and probabilistic analyses indicated that more than the 10-fold of the predicted environmental concentrations would still only lead to negligible effects (mortality< 10%). For the second-most sensitive species, *G. pulex*, deterministic analyses indicated that for moderately increased concentration levels, i.e. up to the 4 fold of the predicted environmental exposure, non-negligible effects can be excluded. For the most sensitive species *C. riparius*, all analyses gave indications that predicted environmental concentration levels would lead to non-negligible effects on survival. It must be critically remarked, however, that as a result of model calibration overestimation of mortality is likely for concentrations larger than 0.2 μg/L, and that quality and quantity of validation data for *C.riparius* did not allow firm conclusions about the realism of predictions. Modelling results show the potential of screening large numbers of exposure scenarios using TK/TD modelling, and how TK/TD models can be used to evaluate realistic time-variable exposure more extensively than by toxicity-exposure ratios alone. The results show that the toxicity potential of an exposure scenario is not governed by the maximum concentration alone.

WE157

Quantification of uncertainty in toxicokinetic-toxicodynamic model predictions

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/ Institute for Risk Assessment Sciences; N. Cedergreen, University of Copenhagen / Plant and Environmental Sciences; V. Ducrot, Bayer CropScience AG / Environmental Safety Ecotoxicology; R.B. Schaefer, University of Koblenz-Landau / Institute for Environmental Sciences; T. Jager, DEBtox Research / Dept of Theoretical Biology; R. Ashauer, University of York / Environment

Toxicokinetic-toxicodynamic (TK/TD) models quantify the expected chemically-induced mortality from any time-variable, environmentally relevant exposure profile (Ashauer et al., submitted), leading to more realistic predictions of individual and population level effects on sensitive species. The flexibility of TK/TD models enables their use for different extrapolation purposes of relevance in risk assessment. As an example the evaluation of potential toxic effects from the defined exposure time series in the FOCUS surface water models (Ashauer et al. 2013). TK/TD models allow directly relating exposure patterns to expected effects, thereby increasing accuracy and decreasing method-associated uncertainty of risk estimates. In addition, TKTD model predictions provide a means for quantifying uncertainty that is associated to biological variability as captured in the TK/TD model parameters. This study shows how information collected during model parameter estimation can be used to analyse uncertainty of model predictions in a probabilistic way. Model parameters were estimated from observed survival data for a set of four pesticides (carbendazim, cypermethrin, dimethoateand malathion), by maximizing the likelihood estimation. To approximate the joint confidence regions of all parameters, those parameter sets from the optimisation procedure that were not rejected in a likelihood ratio test were selected and subsequently used to generate forward predictions. The minimum and maximum of all predictions at each time point can be interpreted as a 95% uncertainty interval on the model predictions (reflecting parameter uncertainty). Model results for the four selected pesticides indicate that the sample size, the quality of data used for model calibration and the properties of the chemical-species interactions (e.g., the speed of the toxicokinetic and toxicodynamic recovery processes) have a direct impact on the parameter estimates, in particular uncertainties, and hence on the precision of model predictions as reflected by the size of the uncertainty bands of survival dynamics plots of and dose-response curves. Deterministic model predictions using best-fit parameters are in this way augmented with information about uncertainty of model predictions. This supports the evaluation of such model predictions for consideration in the regulatory environmental risk assessment of chemicals.

WE158

Variability in life stage sensitivity is a matter of toxicokinetics

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Aquatic effect assessment uses results from short-term laboratory experiments in which it is purposely attempted to keep constant exposure conditions and to minimize inter-individual variability, mainly in the body size or age of the tested animals. In contrast, natural populations are in most species size- or age-structured. Body size is an important demographic attribute, not only because it determines population properties such as abundance and density but also controls the main ecological processes regulating population dynamics such as density-dependence and size-selective predation. Furthermore, individuals in the same population exhibit different sensitivities to the same exposure conditions, depending on their life stage, which means that toxicity might be under or overestimated based on the standard toxicity tests. In this study, we investigated to what extent intraspecies variability in sensitivity can be explained by differential toxicokinetics, i.e. uptake and elimination of the chemical. Furthermore, we tested toxicokinetic-toxicodynamic models of different complexities, including body size scaling approaches, for their ability to represent lethal effects observed for *Daphnia magna* exposed to triphenyltin. To assess the consequences of these approaches at the population level, we simulated different scenarios using an individual-based model and confronted predictions with population data from pulsed exposure scenarios. Our aim is to evaluate the importance of considering population demography in toxicokinetics and toxicodynamics for understanding and predicting potential chemical impacts at higher levels of biological organization.

WE159

Ecological scenarios for the assessment of chemical effects on stream communities: a mechanistic modelling approach

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The ecological risk assessment of chemicals aims at quantifying the likelihood of adverse ecological effects posed on populations and the communities they comprise. Effects caused by the exposure of organisms to toxicants can however to a great extent depend on environmental scenarios as well on the states, behaviors and interactions of organisms with consequences for individual life history, population responses and community dynamics. In the past, these aspects have often been ignored in ecotoxicology and the calculation of adverse effect concentration, which classically focuses on toxicant concentration responses while keeping the environmental conditions as constant as possible. We present the conceptual design of a mechanistic simulation model which aims at the integration of environmental scenarios into the ecological risk assessment of chemicals. This individual based community model will integrate stream typologies as formulated within water framework directive, functional trait data bases, spatial explicit movement behavior, dynamic energy budgets triggering live history processes and toxicokinetic-toxicodynamic models linking exposure and effects in a dynamic manner. We anticipate that this project will increase the understanding of how chemicals act on multiple interacting species and provide a basis for bringing more ecological realism into the ecological risk assessment of chemicals.

WE160

Quantifying the energetic cost of toxicant stress in insects: a full cycle approach

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The standard dynamic energy budget (DEB) model represents a quest for the simplest model that can describe the full life-cycle bioenergetics of all living organisms. Progress toward this goal is made by emphasising similarities between organisms, rather than differences. Such universal life processes include energy acquisition, growth, development, maintenance, excretion and reproduction. However, substantial modifications to this model are required to capture insects in a DEB context. These insects pose several challenges for ontogenetic development modelling, including discontinuous growth due to moulting, non-standard growth curves, lack of growth in adults, and the energetics of metamorphosis. Here we present a simple DEB model for insects that captures energy allocation processes through the egg, larval, pupal, and adult stages. The model is tested against both original and compiled data on growth, development, stoichiometry, respiration, and reproduction for a variety of insects and is shown to predict such data well. We demonstrate that toxic stress induces a range of measurable effects, from increased development time to increased respiration, that can be linked and quantified using our presented framework. Through the mechanistic modelling of fundamental energetic processes, the presented bioenergetics model for insects can be used to better quantify sub-lethal effects of toxic stress at the system level of the organism. The project was financed by the National Science Centre project HARMONIA (No. 2012/06/M/NZ4/00137).

WE161

DEVELOPMENT AND APPLICATION OF GENERIC TOXICOKINETIC MODELS IN FISH TO ENVIRONMENTAL RISK ASSESSMENT OF CHEMICALS

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One of the goals of environmental risk assessment is to protect animal populations and the whole ecosystem from adverse effects resulting from exposure to single or multiple chemicals. In principle, risk assessment methodologies rely on combining estimates of exposure to the chemical(s) with toxicity data (hazard) used for risk characterisation. Exposure assessment aims to quantify “external dose”, as the amount of chemical agent(s) that reaches an organism. Hazard assessment for a chemical aims to quantify the relationship between dose and toxicity so that reference points can be set to derive environmental standards for specific protection goals. Over the last two decades, many research efforts have aimed to improve the quantification of such dose response relationships through the integration of “external dose” and “internal dose” namely toxicokinetic (TK)/ADME (Absorption, Distribution, Metabolism, and Excretion) processes. For this purpose, TK models have been developed to estimate internal doses from external doses in a time dependent manner, characterise the consequence of metabolism to identify the toxic species for a given compound (parent compound/metabolite) as well as extrapolate between laboratory and environmental conditions or between species. The purpose of this project is to develop a generic TK model for fish species to predict internal doses under different environmental scenarios of exposure. The model will be adaptable to a number of teleost fish species and validated by using data for studies on regulated products (*e.g*. pesticides) and environmental contaminants. Extensive literature searches will be performed to review models and tools incorporating TK for environmental risk assessment, collect biological and physiological parameters for given fish species and TK parameters for chemicals of environmental relevance. From this data collection exercise, tools aiming at predicting TK parameters for a number of fish species (*i.e*. interspecies extrapolation) will be developed. These tools will allow the calibration of fish physiologically-based TK (PB-TK) model taking into account several compartments (*e.g*. liver, fat) to model ADME processes for chemicals under different exposure scenarios. Finally, the predictive ability of the model and the uncertainties will be assessed for both TK parameters taking into account fish species differences and prediction of toxicity parameters.

WE162

Using mechanistic effect modelling to justify the TWA approach in aquatic risk assessment

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Risk assessments based on laboratory toxicity tests performed under constant exposure conditions may overestimate potential risks if the exposure concentration of the experiment is compared the PEC_{max} for short term exposure situation. To use PEC_{TWA} for the aquatic risk assessment several criteria have to be assessed following the EFSA Aquatic Guidance document: reciprocity and onset of effects as well as latency of effects. Most of these criteria cannot be assessed based on standard studies alone. On the one hand several endpoints are only measured at the end of the experiment. On the other hand effects are often found only in a few concentrations. Therefore mechanistic effect modelling especially toxikokinetics-toxicodynamics modelling is already recommended in the EFSA Aquatic Guidance document to test these questions. In this poster we will demonstrate case studies in which we used mechanistic effect modelling (namely GUTS and IDamP) to justify the TWA approach compound and species specific. Therefore the models were calibrated to the experiment triggering the risk assessment, if possible the preditive power of the models were tested on a different dataset (model validation). In the last step the models were used to investigate the questions necessary to justify the TWA approach according to the EFSA Aquatic guidance document.

WE163

Landscape context in bee risk assessment - a way to a realistic worst case environmental scenario

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The western honey bee (*Apis mellifera*) is considered one of the most important pollinators concerning food crops. No sole underlying cause for increasing winter colony loss has been found so far, although there is consensus that the issue is of multifcatorial nature, and that multiple stressors, in addition to pesticides especialy landscape characterisitics and parasites play an important role. In the present work a modified version of the BEEHAVE model has been used to understand the effects of pesticides on a single beehive under different ecological conditions. A pesticide module was added to simulate the effects of pesticides on foragers and larvae due to nectar and contact exposure. Virtual experiments are conducted to figure out how landscape context, weather, beekeeper practice and *Varroa* mites affect the outcome of a pesticide risk assessment. Virtual experiments were conducted on different levels of complexity starting with two artifical patches over simplified scenarios up to realistic landscapes. The model approach allows to adress directly the specific protection goals, namely colony survival, colony size (number of bees in a colony) and honey production. We tested in virtual experiments different combinations of beekeeping practice, weather and landscape features on bee colony health with and without the impact of a hypothetical pesticide. This kind of analysis could not be conducted experimentally showing the strength of simulation models like BEEHAVE for environmental risk assessment. As defined in the EFSA Scientific Opinion on Good modelling an environmental scenario is a combination of agronomic, abiotic and biotic parameters which forms a realistic worst case situation. We will demonstrate that if setting up ecological scenarios the baseline against which the pesticide is tested has to be clear and this is not straightforward. To exclude unrealistic scenarios we suggest introducing the following constraint (boundary condition) for a realistic worst case scenario for bee risk assessment: honey harvest of at least 20 kg per hive should be possible within each year otherwise the beekeeper would not place their colonies in this landscape Comparison of simple scenarios with realistic landscapes shows that realistic scenario selection can be possible, but a minimal complexity is necessary for an realistic worst case environmental scenario.

WE164

Colony level impact of sublethal pesticide effects on honeybees: a simulation study using BEEHAVE

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The research on neonicotinoids and honeybees have changed focus from direct mortality to sublethal effects such as reduced mobility, learning, orientation and effects on brood care. Most of these tests have been performed in the lab using methods such as the proboscis extension reflex or histology, which would be challenging to translate into quantifiable effects on the individual's or colony's performance under field conditions. Here we use a published honeybee model, BEEHAVE [1], to study what impact sublethal effects may have on colonies. We combined two crop scenarios (oilseed rape, sunflower) with direct mortality and three sublethal effects (brood care, disorientation, flower handling). Actual effects on individual bees will depend on exposure concentrations, but our purpose was to compare responses so we enforced effect sizes that caused colony level impact. In the oilseed rape scenarios, poor brood care had the largest colony impact as it created a bottleneck for the important spring build-up of the workforce. For all effect types, the impact on number of workers typically peaked about a month after exposure and lasted some time before the colony started to recover. In the sunflower scenarios, the exposure timing was later and that changed the colony's response to the toxic effects. The colony impact now peaked during exposure and the ranking of effects differed with poor brood care now having negligible impact, because the reproductive season was ending. In contrast effects on foragers were more severe because the bottleneck now was build-up of honey stores. In both crop scenarios, good forage mitigated all effect types substantially. We recommend that in field studies colony assessments should continue at least one month after exposure finishes in order to ensure detection of ecologically relevant sublethal effects. Our results indicate, that even if the actual sublethal effect is difficult to detect in the field, subsequent ecologically relevant impact on colony dynamics would be clear if colony assessments are continued and/or if the colony is placed on scales. While sublethal effects may have the potential to impact colonies, evidence from several published field studies indicates that the exposure following seed treatments is not sufficiently high to induce such ecologically relevant effects because no colony level impacts are seen. 1] Becher et al. 2014. *J Appl Ecol* 51:470-482.

WE165

Modelling the effect of bioturbation on pesticide exposure of benthic Chironomus riparius populations

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Hydrophobic pesticides accumulate in sediments and may pose a risk to benthic organisms. Benthic organisms are exposed via pore water and ingestion of contaminated organic matter. The vertical distribution of pesticide in the sediment determines to a large extent the relative importance of both exposure routes, driven by diffusion in pore water, degradation, adsorption and bioturbation. Bioturbation is associated with considerable increase in the vertical transport due to bioactivity but is currently not considered in the EU pesticide risk assessment. The objective of this research is to assess the impact of bioturbation on the survival of a benthic organism, while using a modelling approach. Diepens et al. (submitted) calculated exposure profiles in the upper 1 cm of the sediment (pore water) and the upper feeding layer of 0.2 cm of the sediment (in organic matter, OM) after pulsed chlorpyrifos entries following a standard FOCUS pond scenario. These profiles were linked to a *C. riparius* population model, with individual exposure linked to effects by means of a toxico-kinetics toxico-dynamics (TKTD) model. In this study similar concentration profiles were calculated while including bioturbation as a particle dispersion process in the TOXSWA model. Comparison of chlorpyrifos exposure profiles with and without bioturbation showed that the profiles of pore water concentrations did not deviate much, however, OM bound concentrations were lower in the top 2 mm when bioturbation was considered. The impact on *C. riparius* population exposure was also less than when neglecting bioturbation. Hence making the risk assessment more realistic by taking into account bioturbation of sediment-bound chemicals may change the outcome considerably. A next step will be to include possible feedback mechanisms between population density and bioturbation. *References* Diepens, N.J., J.M. Baveco, W.H.J. Beltman, A.A. Koelmans, P.J. Van den Brink. Dynamics and recovery of a sediment exposed *Chironomus riparius* population: A modelling approach. (submitted)

WE166

Individual-based simulation models of multi-species systems under the impact of chemicals: Complex dynamics can lead to changes in population recovery times and regime shifts

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There is a tendency to include ever more realism in models used for prospective environmental risk-assessment. Incorporating species interactions in multi-species models, may lead to emergent 'complex' dynamics and systems with alternative stable states (ASS). With ASS sudden shifts to alternative state may occur, following disturbances. In a previous study, we analysed complex dynamics in individual-based models (IBMs), combining IBM simulations with analyses of a strategic model. We found that stress could change the stability regime of multi-species models and trigger transitions to ASS. As an example of competing species the aquatic macro-invertebrates *Asellus aquaticus* and *Gammarus pulex* were chosen; the classical Lotka-Volterra competition model (LVCM) served as the accompanying simple, mathematically-tractable, model. Identifying the potential of ASS in multi-species systems emerged as one of the important roles of models. With the same models, we perform additional analyses aimed at further understanding the consequences for risk assessment. Firstly, we identify what might go wrong with estimates of recovery times when these are based on a single-species models, when in the actual, real, system, competitive interactions occur. Secondly, we show how much of the rich behaviour of the system we would 'miss', when we address competition but a-priori limit ourselves to the region in parameter space associated with coexistence. Results show that an observer of the dynamics of *Asellus* only, will encounter a bewildering variety of behaviour, ranging from fast and slow recovery to quick extinction. This variety in dynamics is logically related to the settings chosen for the competitor and easily explained from bifurcation diagrams. We conclude that when competition is playing a role, it needs to be addressed in population models, as it will have a large impact on recovery times. Recovery times depend on characteristics of target and competitor species. When the multi-species system has ASS, this is an additional complicating factor: the relative abundance after a stress event may lead to a regime shift, implying that recovery of the target species will never take place. Considering dynamics only for the coexistence case will ignore an important part of the potential behaviour of a competition system, and thus many possible alternative outcomes for recovery times will not be accounted for. From the results, we formulate a set of recommendations.

WE167
CPFISH: A new approach for evaluation of binary data in many-to-one control vs. treatment setups
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Fisher's exact test for pairwise comparisons of proportions is widespread. However, sequential application induces alpha inflation. Bonferroni correction or Bonferroni-Holm correction of p-values are often used to overcome this issue. For detection of significant effects in binary control vs. treatment setups proportion data are often evaluated by using multiple statistical tests, e.g. Dunnett t-test. While normal approximation of these data is a minor problem multiple t-tests suffer from variance heterogeneity. Applying arcsin-square root transformation variance homogeneity is provided. However, normal approximation is corrupted. To avoid data transformations the Cochran-Armitage-test has been proposed to investigate trends in proportions. The test statistic used is approximately normal. Thus, critical values, p-values and derived significant effects lack of statistical accurateness, too. Other tests based on approximate normal distribution are the maximin efficiency robust test, MAX3, the constrained likelihood ratio test and the GMS. In times of high computational power we can account for alpha inflation by using the closure principle (CP), instead. There is no need for adjustment of p-values. We propose to apply the extended version of Fisher's exact test (FISH) for 2 x c contingency tables in coherence with the CP using R statistic software. The FISH neither makes use of data transformations nor of approximate distributions. Using the real binomial distribution of the data CPFISH does not suffer from the drawbacks induced by normal approximation and heterogeneous variances. By combining CP and FISH the CPFISH is derived. The applicability of CPFISH is shown by means of real and fictitious data sets. Finally, we compare CPFISH to the well-established approaches of Dunnett's multiple t-test in coherence with arcsin-square root transformation and Fisher's exact test in coherence with adjusted p-values using Bonferroni and Bonferroni-Holm correction.

WE168
Species Sensitivity Distributions with Censored Values
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Species Sensitivity Distributions (SSDs) are increasingly requested by regulatory authorities, especially in the EU, as part of product registration submissions. There is some inconsistency among country regulators and EFSA guidance as to requirements and acceptable methods of fitting and interpreting SSDs. The primary issue to be discussed the handling of censored values (e.g., EC50>100 ppm). It has become common in some EU registrations to reject species data for SSD purposes if the data do not conform to the log-normal distribution. There is no scientific basis for this requirement and its use does not serve regulatory conservatism. Indeed, exclusion or improper treatment of censored values tends to increase HC5 estimates. Another issue is forcing all data to fit a single distribution, such as log-normal. It is shown that this too can produce significant bias. The treatment of censored data in SSD fitting has not received as much attention as it deserves, given that right-censored data is common in toxicity studies and occasionally, left-censored values are found. The later are common in monitoring data, but not common in toxicity studies. Some recent regulatory guidance suggests discarding censored values in fitting an SSD. (e.g., EFSA 2014; EFSA 2015; Schmitz *et al*. 2015) or using them only under strict conditions (EFSAS 2013). There is no scientific basis for avoiding censored values or for treating them as uncensored. There have long been known mathematically correct ways to include censored values in fitting a distribution. Ignoring censored values means the distribution being analyzed is truncated and there are well known differences between truncated log-normal and log-normal distributions. The bias introduced by eliminating censored values will be demonstrated, both through datasets used in product registrations, computer simulations, and mathematics. A well-known mathematically correct approach to including censored values will be described. Force fitting a standard distribution or rejecting data that do not conform to this distribution, and exclusion or improper treatment of censored data will bias the HC5 and HC5LB estimates. Mathematically sound and tractable methods for selecting distributions and handling censored values are known and available in validated software. There is no good reason for ignoring sound science in fitting or interpreting SSDs.

WE169
Dynamics and recovery of a sediment exposed Chironomus riparius population: A modelling approach
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To assess risks of sediment-bound contaminants, larger temporal and spatial scales have to be addressed than can be covered in laboratory tests. Although models can address these scales, they usually lack the coupling between chemical fate in the sediment, toxicokinetic-toxicodynamic processes in individuals and the propagation of individual-level effects to the population. We developed a population model that includes all these processes and assesses the importance of chemical uptake routes on damage and recovery of a *Chironomus riparius* population after pulsed chlorpyrifos exposure. We show that particle ingestion is an important additional exposure pathway affecting *C. riparius* population dynamics and recovery. Neglect of particle ingestion underestimates damage and recovery times, which implies that risks of sediment-bound chemicals are underestimated. Additional scenario studies showed the importance of selecting the biologically relevant sediment layer and the use of long term data output.

WE170
An individual-based model of the three-spined stickleback: incorporating effects on breeding behaviour into the assessment of endocrine disrupting chemicals.
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Population modelling is employed to extrapolate from individual effects (including behavioural effects) to population-level effects in the environmental risk assessment (ERA) of chemicals. Population models that incorporate ecological processes such as density dependent competition, individual variability and aspects of behaviour would add further realism to risk assessment. Reproduction in some fish species involves complex breeding behaviours that can be affected by chemical exposure but ERA does not incorporate aspects of behaviour into regulatory testing. In the three-spined stickleback (*Gasterosteus aculeatus*) it has been shown that nest building, courtship displays and parental care, may be disrupted by exposure to endocrine disrupting chemicals (EDCs), consequently affecting population recruitment. Here, we present an individual-based model (IBM) for the three-spined stickleback with the purpose to simulate realistic scenarios for the chronic exposure effects of EDCs. The three spined stickleback is widespread geographically, and potentially sensitive to chronic exposure to EDCs that mimic sex hormones given its complex breeding strategy, low fecundity and the provision of high level of parental care. Density dependent growth and mortality and individual breeding behaviours are key parameters within the model. The IBM has been structured using a series of sub-models, based on empirical data obtained from published literature. The poster will present a full description of the model with some preliminary testing, and illustrate its potential application within ERA.

WE171
Tribolium castaneum under toxic stress: mechanistic effect model for a holometabolous insect
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Various bioenergetics models for animal species are available to address questions about organism functioning under stressed conditions. However, a limited number of such models exists for holometabolous insects, mainly due to difficulties in capturing the complexity of their life cycle. These organisms are characterized by several metabolically distinct developmental stages, *i.e*. egg, larva, pupa and adult. We have developed a bioenergetics model to describe the entire life cycle of the red flour beetle *Tribolium castaneum* (Coleoptera: Tenebrionidae) under toxic stress. Experiments in which individuals were exposed to copper concentrations over 90 days were conducted. The obtained experimental data on individual

survival, growth, development and reproduction over time were used to calibrate the model. The model shows good performance at describing the life cycle of an individual organism. Although the beetles undergo a complex developmental path, our model captures well the pattern of toxic effects such as impaired development or reproduction. We demonstrate how conducting one integrative analysis, based on combined modelling and experimental approaches, may provide a valuable mechanistic insight into the energy budget of the red flour beetle under toxic stress. Finally, aiming at being generic for holometabolous insects, this model has a potential to address issues relevant for chemical risk assessment of regulatory important organisms such as bees or non-target arthropods. The project was financed by the National Science Centre project HARMONIA (No. 2012/06/M/NZ4/00137).

Quantitative in vitro to in vivo extrapolation (QIVIVE): Advances in tools to quantify exposure (dose)-response relationships and use in risk assessment (P)

WE172

How representative are rat liver cytochrome P450s for possible endocrine disruptors identification? An in vitro azoles screening study.

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Endocrine disruptors are a group of chemicals that interfere with the endocrine system and are an emerging health concern. In this study we focus on the endocrine disrupting chemicals that inhibit the enzyme aromatase (CYP19) in the steroidogenesis and therefore create imbalance in the vertebrates' hormone systems. CYP19 belongs to a larger group of enzymes called cytochrome P450 monooxygenases, which are preserved across different species. They are heme-containing metalloenzymes that catalyze hydroxylation and oxidation on the metabolism of thousands of endogenous and exogenous compounds. Azole antifungal agents are designed to inhibit 14α-demethylase in the fungal ergosterol biosynthesis and therefore they act as inhibitors for other non-target cytochromes as well. In this study, 21 different azoles were tested *in vitro* on rat liver microsomes (ECOD) activity and compared with previous studies on CYP19, in terms of their inhibition potential. The rat liver microsomes were used to represent the cytochrome P450s enzymes and. There was a positive correlation (imidazoles a=0.34, R^2= 0.70 and triazoles a=0.68, R^2=0.52) between P450s and CYP19 50% inhibitory concentrations (IC50) suggesting that azoles were stronger inhibitors of P450s as compared to CYP19. Imidazoles appeared to be more potent than triazoles for both P450 and CYP19 inhibition. The structure and the lipophilicity of the compound play an important role for the affinity of the inhibitor for the enzymatic binding pocket. It was shown that inhibition, measured as IC50, increased with increased lipophilicity of the compound. For measuring P450 activity 7-ethoxycoumarin was used as a standard substrate because of its low selectivity. Testing different substrates such as testosterone can possibly be more relevant to compare to CYP19 activity. The results of this study are encouraging on further studying the possibility to use rat liver microsomes as representative for human CYP19 and as a screening tool for possible endocrine disruptors.

WE173

Critical Membrane Concentrations of Amines in In Vitro and In Vivo Fish Bioassays

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Neutral organic contaminants are assumed to be nonspecifically toxic at the baseline critical membrane burden (CMB) of ~100 mmol/kg lipid. Assuming a contaminant's octanol-water partition coefficient (logP) is equal to its membrane affinity (logKmw), the specificity of the toxic mode of action (or 'excess toxicity') can be estimated, where CMB in mmol/kg lipid = median lethal concentration (LC_{50}) in mmol/L x logKmw in L/kg lipids. This assumption needs to be refined for contaminants that are predominantly present in their ionic form in toxicity assays as the CMB has only been determined for a large set of neutral contaminants. This is an important data gap in environmental risk assessment because the majority of pharmaceuticals and a substantial fraction of industrial chemicals are highly ionizable. The aim of this study was two fold: 1) to define the CMB for strong bases (pKa>9) using existing *in vivo* fish acute toxicity data from the US EPA Fathead Minnow Database, and 2) to determine whether this definition could be replicated in an *in vitro* cytotoxicity assay with a fish gill cell line (RTgill-W1). Membrane affinities for a series of amines listed in the US EPA's Fathead Minnow Acute Toxicity Database were determined using immobilized artificial membrane chromatography (IAM-HPLC), which allows for a direct interpretation of the critical membrane burden (CMB) in acutely exposed fish for these cationic chemicals. Accordingly, the CMB of thirteen amines with an unsure mode of action (MoA) was found to be within a very narrow range of 7.6±5 mmol/kg lipid, more than an order of magnitude lower than the baseline CMB for neutral chemicals. For the neurotoxic amines, the CMB of amphetamine was similar to those of the "unsure MoA" amines, while strychnine and nicotine showed an "excess amine toxicity" of a factor of 10, with CMB's of 0.6 mmol/kg lipid. RTgill-W1 cells were exposed for 48h to the abovementioned amines and cytotoxicity was assessed using the Alamar Blue assay. Test media were sampled before and after exposure and amine concentrations were analytically measured to define actual exposure concentrations. Unlike for neutral organic chemicals, *in vitro* median effect concentrations (EC_{50}) for the tested amines were at least twofold higher than fish acute LC_{50} values. This suggests that the specific mechanisms by which amines accumulate or cause toxicity *in vivo* are not replicated *in vitro*.

Application of a coordinated OMICS research program and data into regulatory frameworks: case-studies and perspectives (P)

WE174

Developing an enhanced experimental work-flow for maximising the use of 'omics data within the Adverse Outcome Pathway framework

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Developments in 'omics technologies have opened new possibilities for assessing the molecular and biochemical responses of organisms to chemicals. Feature selection tools are increasingly being used to discover responses within the 'omics datasets that may be predictive of adverse outcomes. These molecular responses can help to define the "key events" (KEs) underpinning the toxicity pathway within an Adverse Outcome Pathway (AOP) framework. KEs are deviations from a healthy state, through a stress response, that may predict an adverse outcome and therefore indicative of a chemical's potential health hazards. Here we investigate the potential of multi-omics technologies to discover KEs of specific acting chemicals and baseline narcotics. The first phase of this work has been the development of a proposed work-flow of how to utilise the non-targeted, information rich, data attainable from omics investigations; incorporating this into an enhanced AOP driven risk assessment approach. The second phase has been to start to develop comparative multi-omics approaches as part of an integrated (weight of evidence) approach in combination with available *in silico* or *in vitro* data to support the categorisation of chemicals by their KEs based upon their molecular and biochemical responses in *Daphnia* and algae. We aim to achieve this by elucidating the relevant KEs of specifically acting chemicals and baseline narcotics and measuring the strength of the association with the subsequent adverse outcome. The use of organisms that span two trophic levels could enable qualitative assessment of the predictive capability of the KE(s) both within and across species, improving toxicity predictions for untested species and in setting exposure thresholds in environmental risk assessment. To identify target homology and further enhance cross species applicability, molecular target sequence analysis tools; such as the US-EPA developed SeqAPASS (Sequence Alignment to Predict Across-Species Susceptibility), will be utilised. Sampling at high temporal resolution (spanning the acute and chronic experimental stages) for the 'omics assays will provide reproducible toxicological effects measurable by gene expression and metabolite profiling. By detecting the point of departure from a healthy state, we aim to use acute exposure scenarios to predict chronic outcomes, ultimately streamlining the experimental design to capture just the KEs making the work flow rapid and cost-effective.

WE175

Transcriptome network analysis of toxicity pathways in aquatic organisms

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Network analysis is based on the guilt-by-association principle and it is a powerful method for identifying patterns within many types of data (e.g. social, medical, biological, environmental etc.). Aiming to expand our understanding of toxicity pathways in aquatic organisms, we developed *neτoxi* – an integrative systems biology workflow, which infers mutual information co-expression networks from large collections of transcriptome data in several organisms (e.g. green algae, zebrafish). Although the biological processes for some chemical stressors have been characterized, most toxicity pathways and their mechanisms of action at the cellular level are still unknown. Integrative bioinformatics and statistical analyses of large and diverse collections of omics datasets have great potential in revealing the mechanisms of cellular response of aquatic organisms to chemical stressors. Specifically, gene co-expression network analyses can identify hubs and modules of genes with similar expression patterns, indicative for structured, modular toxicity response characteristic to certain chemical stressors. *neτoxi* uses both in-house generated omics datasets as well as publicly available data, summing up to more than 650 transcriptome samples in *Clamydomonas reinhardtii*. An entropy based mutual information metric is then used to infer statistically significant co-expression relationships between gene products (i.e. transcripts). The resulting network is visualized as a graph (nodes represent genes and edges show co-expression relationships between genes) and various network statistics are employed to identify interesting patterns such as hub genes (frequently essential genes) and highly interconnected modules (indicators for groups of genes involved in the same cellular process and/or metabolic pathway). Our vision is to use *neτoxi* to identify models of toxicity pathways responses and to advance from single mechanism of action to toxicity pathway-based understanding of chemical effects on the transcriptome of aquatic organisms.

WE176

Ecological case studies using chemical, target and outcome-based approaches to AOP development.

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While formal guidance documentation exists for describing Adverse Outcome Pathways (AOP), development strategies are lacking. Here, different approaches for developing AOPs are considered, including: chemical-, target-, and outcome-based. The chemical-based approach to AOP construction is common. Often, a chemical with a well-studied mode of action is used to identify key events and propose relevant adverse outcomes. An example from the AOP knowledgebase (aopkb.org) is AOP 4: Ecdysone receptor (EcR) activation leading to mortality in Daphnia magna, which is based on the action of the EcR agonists 20-hydroxyecdysone, ponasterone A, and tebufenozide. Prioritization efforts can also drive chemical-based AOP approaches. For example, an AOP describing potential fluoxetine effects on aquatic species was developed because this anti-depressant commonly occurs in aquatic environments. For this effort, the U.S. EPA ECOTOXicology database, which captures single chemical toxicity data, was queried for the most sensitive endpoint (movement) and species (bivalves) to inform AOP 97: 5-hydroxytryptamine transporter inhibition leading to increased predation. Alternatively, target-based approaches to AOP development are widely used for the ongoing OECD effort to catalogue AOPs "that will enhance the utility of US EPA ToxCast high throughput screening data for hazard identification," including AOP 95:ERG channel inhibition leading to reduced survival. This AOP was developed considering a ToxCast assay which measures the inhibition of the ERG potassium channel, an ion channel essential for cardiomyocyte repolarization. After literature review of adverse effects from ERG inhibition, and analysis of protein target conservation across species, an AOP for effects in fish was constructed. Finally, examples of outcome-based approaches for developing AOPs are scant, although they frequently offer stronger weight of evidence for effects at the individual, population and ecosystem levels. For example, AOP 138: PAH channel inhibition leading to mortality, was developed largely from a single study that described dramatic declines in vulture populations. This study linked observations of renal failure and visceral gout in vultures to diclofenac exposure upon scavenging treated livestock. Subsequent studies provided additional information for early key events, including the ability of diclofenac to inhibit uric acid transport by the p-amino-hippuric (PAH) acid channel.

WE177

Environmental effects of cyanobacterial blooms on fish: a Metabolomic approach from experimental fish to natural population.

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Due to the interactive effects of the eutrophication of aquatic ecosystems and global warming, toxic cyanobacterial blooms have become a major scientific concern. They are responsible of the production of a wide variety of toxic secondary metabolites implicated in various impacts on the stability and the functioning of aquatic ecosystems. The Microcystins (MCs) and more generally toxic cyanobacterial metabolites have been implicated in various negative effects experimentally induced on various fish species.\nRecently, with the development of "Omic" sciences, proteomic and metabolomics analyses, using nuclear magnetic resonance (NMR) and/or mass spectrometry (MS), have been shown to provide a powerful tool for the discrimination of metabolic responses between organisms exposed to different treatments and for the identification of metabolic pathways involved in toxicological mechanisms of various toxic compounds. However to our knowledge, there are no such investigations dealing with the global metabolic effects of toxic cyanobacterial blooms on fish using metabolomics analysis.\nIn this way, liver metabolome profiles were analyzed on four typical fish species of freshwater European lakes (perch, roach, crucian carp and pumpkinseed sunfish) exposed or not to cyanobacterial blooms, and originating from both field enclosures, mesocome experiments and field sampling. The aims of this study are (1) to investigate and identify the metabolic changes which may be observed in the different fish species according the presence of cyanobacterial blooms; (2) to assess and identify the potential different responses of the different fish species metabolomes under stress conditions due to a cyanobacterial exposition. These results originating from multi-approach observations will help us to better understand the genuine ecotoxicological effects of cyanobacterial blooms producing a wide variety of potential noxious metabolites on fish populations from natural ecosystems.

WE178

Use of the fresh mussel Dreissena complex as a tool for ecotoxicological studies: development of genomic tools

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Various species of aquatic invertebrates are commonly used as sentinel organisms for assessment of the effects of environmental pressures on aquatic ecosystems through the measurement of biomarkers. In freshwater ecosystems, numerous studies focused on *Dreissena polymorpha* (Pallas 1771), also known as zebra mussel. *D. polymorpha* is an invasive species originated from Ponto-Caspian region, which spread around the world and is now largely represented in Europe and North-America. In the meantime, the closely related quagga mussel *Dreissena rostriformis bugensis* (Andrusov 1897) became in turn invasive in North America and Western Europe where first observation of quagga mussels was made in 2006 in the Hollands Diep. Molecular genetic markers have then been developed to differentiate *Dreissena* species but such mitochondrial genetic tool (COI-RFLP) doesn't allow to identify potential interspecific hybrids. Zebra and quagga mussels disclose physiological differences such as temperature and salinity tolerance, byssal thread attachment, growth, respiration rates, assimilation efficiency and reproduction. We initiated the characterization of the transcriptomes of both species using NGS technology (Illumina sequencing) to 1) identify new nuclear genetic markers that could be used to clearly identify species and/or hybrids (mainly SNPs), 2) develop a genomic database for *Dreissena* complex and generate a large set of candidate genes showing an strong importance in ecotoxicological studies and 3) generate a SNP database for polymorphism studies. Results presented here describe the main differences observed in two tissues transcriptomes (gills and digestive glands) between the two species studied and gives previous information on putative SNPs that could be used for species identification. We also explicit the main physiological functions identified and highlight set of candidate genes suitable for ecotoxicological purpose.

WE179

Constitution of a reference transcriptomie resource for the copepod Eurytemora affinis and differential expression between sexes exposed to acetone

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Copepods represent the most abundant group of aquatic invertebrates which includes freshwater and marine species. Among them, the calanoid copepod *Eurytemora affinis* is widely represented in the northern hemisphere estuaries and has become a species of interest in ecotoxicology. Like other non-target organisms, *E. affinis* could be exposed to a wide range of chemicals. In this context, we investigated the transcriptome of *E. Affinis* in order to (i) constitute a bank of sequences for *E. affinis* and (ii) highlighted sex-specific patterns in *E. affinis* exposed to solvent acetone as a base for future chemical exposure. After 3 days of stalling, a first sample of 500 copepods (including males, females and young stages) was realized. Then, a solvent (acetone, 0.005% w/v) exposure was performed. After 48h of exposure, 400 males and 400 females were sorted and frozen for further analyses. RNA from non-exposed and exposed organisms were respectively sequenced by 454 GS FLX and Illumina®. *E. affinis* transcriptome was functionally explored using the KEGG pathway database. The differential expression analysis was performed from the acetone samples. Differentially expressed genes (DEG) were investigated after applying a threshold of PPDE (Posterior Probability Differentially Expressed) of 0.95 and a log2(FoldChange) $\geq$ 2. A total of 1,651,761 reads were pyrosequenced. *De novo* assembly resulted in 16,903,584 bases divided in 19,721 transcripts with a mean length of 857 pb. About half of them (9,729) were annotated. 64% of the Blast results were provided by arthropod sequences including crustaceans and insects. 6,446 sequences were identified in 321 pathways. Among them, the category "cellular processes", "environmental information processing", "human diseases", "organismal systems", "genetic information processing" and "metabolism" were illustrated. Moreover, 1,786 DEG were identified between sexes after acetone exposure highlighted sex-specific patterns. 1,072 genes were expressed in females whereas 714 genes were expressed in males.

WE180

Sorting the signals: meta-analysis of transcriptome data of zebrafish embryos after chemical exposure

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Adverse effects of chemicals on cells and organisms are in many cases either preceded by or result in gene-expression changes (Nuwaysir et al., 1999). This makes transcriptome analysis a promising tool in ecotoxicological research, e.g. as future bioassay or diagnostic tool for biomontioring. There are several advantages in comparison to established tools, e.g. the non-targeted approach, allowing assessment of several modes-of-action at the same time, or the possibility to combine assessment of adverse effects with identification of effect-drivers, which could also be helpful in the establishment of adverse outcome pathways (AOPs). During recent years several studies analysed global gene expression in *Danio rerio* embryos after exposure to chemicals (Williams et al., 2014). Many of those studies show specific gene-expression changes in zebrafish embryos after exposure to chemicals and claim to offer a proof of concept of the suitability of microarray analysis as potent bioassay. To put the so far published results into context, and analyze the quality and comparability between the studies, we performed a meta-analysis of published transcriptome data. The analysis combined the data of 33 published studies using different chemicals, concentrations and exposure times. The analysis revealed a large heterogeneity in gene expression profiles. In contrary to our expectations no gene could be identified as regulated across all conditions. For sure, heterogeneity can be partly explained by differing experimental designs and the lack of time- and dose resolved data. Since comparability is one major requirement for any OMIC-technology on the way to usability in risk assessment, we propose an experimental design for transcriptomics with time and concentration resolved measurements. Nuwaysir, E. F., Bittner, M., Trent, J., Barrett, J. C., & Afshari, C. a. (1999). Microarrays and toxicology: the advent of toxicogenomics. Molecular Carcinogenesis, 24(3), 153–9. Williams, T. D., Mirbahai, L., & Chipman, J. K. (2014). The toxicological application of transcriptomics and epigenomics in zebrafish and other teleosts. Briefings in Functional Genomics, 13(2), 157–71.

WE181

The psychotropic family of pharmaceutical impact the freshwater organisms

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Aquatic organisms are frequently exposed to a wide diversity of chemical compounds suspended or absorbed on substrates. These compounds such as pharmaceuticals are released into aquatic environment from either industrial or domestic effluent. Most of chemicals, especially psychotropic pharmaceuticals, are very persistent even after biotic and abiotic treatments in environment and waste water treatment plant (WWTP). Despite of the important occurrence of detection, few study concern the aquatic environmental impact. Among the aquatic biodiversity, are the molluscs, broadly studied for their grade in the food chain (primary consumer), but also because of their low celerity. *Radix balthica* is a freshwater mollusc present in many French rivers, allowing easy sampling of this snail for eggs collection. The flatworms including *Schmidtea polychroa,* belong to benthic invertebrate and are also studied as ecotoxicological model due to their range in the food chain. Dugesidae is a genus broadly studied, first for their ability of regeneration and secondly for their range in the food chain (secondly consumer). The aim of this study was, to evaluate the toxicity of four psychotropic drugs from four different therapeutic classes and three chemical families. Oxazepam (anxiolytic, benzodiazepine), carbamazepin (antiepileptic, benzodiazepine), cyamemazin (neuroleptic, phenotiazin) and sertralin (antidepressant, Inhibitor Selective of Reuptake of Serotonin (ISRS)) have been selected because of their frequencies and their presence in the French WWTP effluents. Pharmaceuticals toxicity was evaluated on *R. balthica* embryos using hatching success and hatching delay on a concentration range from environmental concentration to 100μg/L. For instance, these indicators showed an effect from 1μg/L to 100μg/L for oxazepam. A differential RNAseq method and an RT-qPCR validation were released on *R. balthica* exposed of oxazepam at 10μg/L and 0.815μg/L or to reconstituted water for control. Statistical analyses have shown 144 contigs differentially expressed for the 10μg/L condition. Among these differentially expressed contigs, we have highlighting several pathways impacted. Pharmaceutical impact was also evaluated on the movement, reproduction ability and regeneration integrity of *Schmidtea polychroa*. Only the movement and the reproduction results showed significant differences to the control. The RT-qPCR study is in progress for targeting the transcriptomic responses relating to the phenotypic responses.

WE182

A Combined Targeted/-non-Targeted Metabolomics approach using UHPLC-Oribtrap- MS: Method validation and ecotoxicological applications

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Characterization of the naturally occurring small molecules in a biological sample(i.e. the metabolome) has shown great promise in ecotoxicology for characterizing chemical modes-of-action, fingerprinting toxicant exposure, or redefining low dose effects levels. Metabolomics can be carried out using either a targeted approach, involving a full complement of standards, or non-targeted approach, in which analysis is carried out in open scan and individual peaks are identified through massspectral library\nmatching. Advantages of the former include generation of quantitative data and improvements in accuracy and precision, while the latter offers the possibility of discovering novel biomarkers.Few have attempted to combine these approaches in order to quantify known metabolites,while also capturing data on novel biomarkers. \nHere we report on the development of a novel targeted/non-target metabolomics approach using ultra-performance liquid chromatography-high resolution (Orbitrap) mass spectrometry.The method facilitates targeted, quantitative analysis of 20 amino acids, 10 non-proteinogenic amines, 7 biogenic amines, 7 TCA-intermediates, and 10 nucleobases and neurotransmitters in multiple reaction monitoring mode, while simultaneously collecting high resolution (Rs>100,000), accurate mass (< 1ppm) data in open scan (m/z100-1000) to facilitate novel biomarker ID. Three methods were developed to maximize metabolite coverage: The first 2 methods employed C18 (1.8μm, 2.1x 100mm) and HILIC (1.7 μm,2.1 x\n100mm) stationary phases with trifluoroacetic acid and acetonitrile mobile phases for determination of non-lipid metabolites.The third method utilized flow injection-MS/HRMS with isotopic overlap deconvolution for determination of >150 lipids (including carnitines, glycerophospholipid and\nsphingomyelins). The benefits of HRMS for FI-MS/MS-based lipidomics were assessed by comparing results with those obtained using the identical method run on a quadrupole mass spectrometer. Non targeted data for all analyses were processed using TracMass2 and MetaboAnalyst. This method is among the first to combine a combined targeted/non targeted approach to obtain quantitative information on known metabolites, while offering the possibility of novel biomarker ID.

WE183

Metabolite formation from 3-methylindole by hepatic microsomes from carp (Cyprinus carpio) and rainbow trout (Oncorhynchus mykiss) during phase I metabolism

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Emission from intensive livestock can affect health of living organisms. The presence of 3-methylindole (3MI), a substance found in mammalian faeces, in contaminated groundwater migh negatively affect physiological processes in fish. Currently, no information is available on 3MI metabolism by fish. We investigated 3MI metabolism by two fish species, carp and rainbow trout, at two ages. Hepatic microsomes from fish were exposed to four concentrations of 3MI (from 2 to 100 μM). Rainbow trout produced two metabolites, 3-methylindole (3MOI) and indole-3-carbinol (I3C), while carp produces only 3MOI. The rate of 3MOI production was similar in both species at both ages. The rates of metabolites formation from 3MI in the presence of specific chemical inhibitors indicated that the CYP450 isoforms CYP1A and CYP3A are mainly responsible for 3MI metabolism. In summary, 3MI is metabolised in fish livers to 3MOI and I3C by CYP450, and formation of these metabolites might be species-dependent. Keywords: 3-methylindole; 3-hydroxy-3-metyloxindole; indole-3-carbinol; cytochrome P450 Acknowledgement - This work was financially supported by the Ministry of Education, Youth and Sports of the Czech Republic – projects “CENAKVA“ (No. CZ.1.05/2.1.00/01.0024) and “CENAKVA II“ (No. LO1205 under the NPU I program), by the Grant Agency of the University of South Bohemia in Ceske Budejovice (No. 012/2016/Z), by the Czech Science Foundation (No. 15-04258S), and by a research grant from C.F. Lundströms Stiftelse, Sweden. The authors thank Dr. Carl Brunius for the synthesis of 3-hydroxy-3-metyloxindole.

WE184

Application of transgenic zebrafish in adverse outcome pathway analysis: a case study on bisphenol A

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More than 1000 chemicals are known, or suspected, to interact with hormone receptors, and/or to affect hormone metabolism[1], and some of these molecular initiating events (MIEs) may lead to adverse effects on a wide range of developmental processes and physiological functions. However, only a small fraction of chemicals have been investigated for endocrine disrupting effects in intact organisms[2]. Although *in silico* and *in vitro* tools provide some indication of the potential endocrine activity of chemicals, reliable *in vivo* systems are required to assess for adverse effects. Transgenic (TG) animal models (e.g. zebrafish) provide *in vivo* systems, in which MIEs triggering adverse outcome pathways (AOPs) can be identified and quantified by fluorescent protein reporters linked to specific receptors or enzymes. Spatial resolution of MIEs at the organ and tissue levels in larval TG zebrafish facilitates targeted assessment of downstream molecular events and subsequent physiological and functional responses. Here we present a case study on embryo-larval TG zebrafish exposed to the estrogenic plasticiser bisphenol A (BPA) previously reported to affect brain development and function at or below aqueous exposure concentrations (230 μg/L) leading to tissue concentrations (^{3}10 μg/kg), which are equivalent to mean human placental concentrations and maximum daily doses for bottle fed infants[3,4]. Using TG(ERE-Casper) zebrafish, which combine a reporter system for indicating Estrogen Response Element activation throughout the body[5] in a translucent Casper phenotype, we show that BPA (100, 1000 μg/L) and its metabolite MBP (0.25, 2.5, 25 μg/L) target the heart valves, with consistent ERE expression at 4 and 14 days post fertilisation (dpf). Via assessments of differential gene expression (using RNA-seq) in micro-dissected heart tissues obtained from treated and control fish at 4 and 14 dpf, and a series of cardio-vascular and other functional endpoints (e.g. heart beat rate, blood flow, critical swimming speed at 14 dpf), we attempt to define AOPs for the two test compounds. References: 1) TEDX 2015. http://endocrinedisruption.org/endocrine-disruption/tedx-list-of-potential-endocrine-disruptors/overview 2) WHO/UNEP 2012. ISBN: 978-92-807-3274-0; 978 92-4-150503-1. 3) Saili et al. 2012. Toxicology 291(1-3):83-92. 4) Kinch et al. 2015. Proc Natl Acad Sci USA 112(5):1475-1480. 5) Lee et al. 2012. Environ Health Perspect 120(7):990-996.

WE185

Proteomic analysis of the estuarine copepod, Eurytemora affinis, in response to endocrine disruptors

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Aquatic ecosystems constitute the chemicals' final destination. One of the chemicals' families targeted by the EU Water Framework Directive is pesticides due to their endocrine disruption (ED) potential. Nowadays, ED effects of chemicals are well documented in vertebrates. Few data are available on invertebrates whereas they represent 95% of the wild fauna. In this context, we investigated the proteomic responses of the widespread copepod *Eurytemora affinis* to model pesticide exposures, in order to (i) improve the knowledge on their effects in crustaceans and (ii) evaluate the potential of proteomic analysis to develop molecular biomarkers of endocrine disruption. After 3 days of stalling in the laboratory, copepods were divided into three 10-liter aquariums. Copepods were exposed to sublethal concentrations of pyriproxyfen (PXF, 10μg/L) and chlordecone (CLD, 10μg/L) separately and in binary mixture (PXF; CLD, 2.5 μg/L; 3.75μg/L respectively). A solvent (acetone 0.005% w/v) control was used. After 48h of exposure, males and females were sorted and frozen for further analyses. After extraction, proteins were identified by HPLC coupled to mass spectrometry (GC-MS-MS). Analyses were performed for both genders for each contamination in order to identify sex-related responses. 1,092 proteins were identified using the GC-MS-MS procedure. The cluster analysis highlighted two groups corresponding to gender. Male and female copepods were specifically more impacted by PXF and CLD respectively. Finally, 76 proteins were differentially detected between conditions including 25 proteins under-expressed and 51 over-expressed. Proteins were involved in several processes such as energetic metabolism and defence. We have focused our investigation on proteins involved in reproduction, growth and development in response to EDs. For example, proteins involved in reproduction like vasa or thioredoxine were under-regulated in male copepods highlighting a potential disruption of gametogenesis. Furthermore, muscle activity with actin and myosin were under-regulated in male copepods, suggesting an impact on locomotion

WE186

Screening of pesticides, alone and in binary mixtures, for ecdysteroid hormone agonist and antagonist activity using the Drosophila cell-based (S2) reporter assay.

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Aquatic ecosystems constitute the chemicals' final destination. One of the chemical families targeted by the EU Water Framework Directive is pesticides due to their endocrine disruption (ED) potential. Nowadays, ED effects of chemicals are well documented in vertebrates and the Organization for Economic Co-operation and Development (OECD) recommends the use of human receptor assays for the screening of ED chemicals. Few data are available on the ED effects on invertebrates whereas they represent 95% of the wild fauna. In this context and in order to explore the effect of endocrine disruptors on invertebrate metabolism, we investigated the potential agonist and antagonist effect on the ecdysteroid receptor for five pesticides known as endocrine disruptors, alone and in a binary mixture using an in vitro test.\nIn this project we used chlordecone (CLD), vinclozolin (VZ), tributyltin (TBT), pyriproxyfen (PXF) and methoprene (MP), as vertebrate and invertebrate models of endocrine disruptors. Of each product a range of different concentrations (from 1.5.10-1 M-1 to 3.10-8 M-1) was tested for investigating their agonist/antagonist potential. Drosophila S2 cells were firstly transfected with the pERE-GFP-ERE-Luc construct composed of ecdysteroid hormone response elements and the reporter luciferase gene. Then the agonist and antagonist activities were tested in 96-well microplates.\nNo agonist activity was observed for each compound alone. Cell toxicity appeared for the highest concentrations. An antagonism was detected for PXF and MP. As juvenile hormone agonist, interaction with the ecdysteroid receptor could be expected. Moreover, CLD – known as an estrogen receptor agonist – may present a weak antagonist activity. TBT did not act as an antagonist of the ecdysteroid receptor. No agonism was observed for any binary mixture and toxicity appeared for the highest concentration. All the binary mixtures showed an antagonist activity. However, interaction between chemicals could induce a response which could be not specific to the ecdysone receptor.\nIn conclusion, the cell-based reporter assay for screening ecdysteroid receptor agonists/antagonists highlighted antagonist activity for both vertebrate and invertebrate models of endocrine disruptors. Binary mixture showed also antagonist activity. However, this response could be non-specific to the receptor. The usefulness of in vitro tests is discussed in relation to predict activities of mixtures in the ecosystem.

Epigenetic and evolutionary effects of pollutants: new challenges for long-term ERA (P)

WE187

Changes in DNA methylation in response to cyanobacteria in the model crustacean Daphnia

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Despite the recent developments in molecular technologies, little attention has been given in environmental toxicology to epigenetic modifications and their potential consequences for environmental risk assessment. We focused on the crustacean *Daphnia* whose well-known ecology and phenotypic plasticity offer unique opportunities to enhance our understanding of epigenetic mechanisms and their importance in environmental challenges. We characterized the methylation pattern in *Daphnia* in response to a rich diet and a diet contaminated with the toxic cyanobacterium *Microcystis aeruginosa*, a common harmful algal bloom known to have detrimental effects on higher organisms. We used bisulfite sequencing to identify methylated cytosines in genomic features. Methylation was primarily targeted to exonic regions while introns and intergenic regions were generally devoid of methylation. We observed that despite the low methylation level in *Daphnia,* some genes are preferably methylated with methylation levels higher than 50% while others have no methylation at all. Roughly 300 genes were differentially methylated between the two treatments. This geneset included ribosomal proteins and RNA polymerases as well as histones and methyl-binding proteins suggesting a functional role for DNA methylation in gene regulation. Overall, the results suggest that DNA methylation may play a significant role in environmental stress responses in *Daphnia*.

WE188

Transgenerational effects of a chronic external gamma irradiation on Daphnia magna: epigenetic modification and inheritance

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Aquatic and terrestrial ecosystems are exposed to radionuclides through planned discharges or accidental releases linked with the nuclear industry activities. Over the past decade, ecosystems protection against ionizing radiation has become a growing public, regulatory and scientific concern. Nowadays, ecological risk assessment for ionizing radiation is still based for a vast majority on studies investigating effects under acute doses and short exposure time, far from realistic environmental conditions in which natural biota are actually exposed. Recently, studies in daphnids at chronic low doses have been conducted over several generations in order to increase our understanding of the long-term effects of low doses of gamma radiation and explore underlying molecular mechanisms. In this context, radio-induced epigenetic modifications have never been investigated in invertebrate species although it was shown that ionizing radiation can induce changes in DNA methylation patterns and gene expression. Epigenetic changes imply the possibility that modifications might be inherited across generations. The present study (still in progress) examines the potential transmission of epigenetic alterations from gamma irradiated daphnids to their non-irradiated progeny up to the third generation (F1, F2, F3). Using the cladoceran crustacean Daphnia magna as a model organism, our objectives are threefold: (i) to examine whether environmentally relevant doses of gamma radiation (0.01 and 35.4 mGy.h-1) received by parents will induce effects on survival, growth and reproduction over non-exposed generations, (ii) to test possible epigenetic modifications caused by gamma irradiation and its transmission to non-exposed generations, (iii) to compare responses to chronic gamma radiation between the molecular and organism levels.

WE189

Crossing scales: From genes to the phenotype and back in ecotoxicological extrapolation for genetic and epigenetic effects - the next frontier

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Proper Science (PS) relies on methods and methodologies that provide tools to extrapolate findings across varying scales. Scales can include organisms, environments, space and time. In Ecotoxicology and Climate Change Science, the issue of extrapolation presents a pivotal challenge to assess the risks of chemicals and other stressors in the environment. Evolution acts on scales different to the genetic and epigenetic effects of toxicants. As a consequence, extrapolation is a formidable temporal problem that may determine speciation, defined as in Hubbell (2001), and furthermore disrupt the genetic diversification triggers. Moreover, assessing the risk for chemicals from the environment remains a debatable target of modern approaches to risk assessment. In our poster we introduce the Daphniapolator, a model that allows transiency of temporal and spatial boundaries to assess risks on selected scales by the user from genes to ecosystems. We demonstrate the feasibility of our approach for an emerging contaminant, next generation carbon nanotubes (NGCT), extrapolating from epigenetic risk to whole ecosystems. The predictive toolbox of the model is based on closed-form solutions of the elementary stochastic differential equations (ESDE), as well simulation techniques including Levy jump process, variance reduction techniques, and pseudo-regression approaches with Martingale stochastic processes (with non-normalized semi-Gaussian regressors). Calibration data originates from population genetic studies in the wild with collembolans under exposure from sewage treatment plants estimating no consequence effect concentration (NCEC) using a reverse Bayesian spatially and temporally explicit model (RBSTM). Data were collected using good laboratory practice (GLP) procedures. We discuss options for extrapolation to other species, across time scales and alleles in the gene pool. We conclude that our model approach represents a very promising approach to unite current challenges in ecotoxicology regarding genome, epigenome, proteomics, metabolic extrapolation problems and issues of pre-selected inference under toxicant stress. Future studies should scrutinise the model application for different problems, scales, compounds and calibration data.

WE190

Development of an OECD two-generations reproduction test in Daphnia magna.

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Most eco-toxicological test guidelines only evaluate the effects observed within one generation, thus disregarding those potential detrimental effects that may appear across generations. The toxicological potential of contaminants may either decrease or increase across generations, or even remain unchanged. Reproductive toxicity in mammals monitors the effects on the parental generation as well as on their offspring. In fact, there are already standardized test procedures aimed to assess multigenerational effects for mammals and fish, but there are no equivalent tests for invertebrates, despite that their short life-cycles offer to cost-effectively monitor multigenerational effects of toxic substances in individuals and populations. Here we described the activities that are currently been conducted on behave of the SETAC EVOGENERATE Working Group to set up protocol for a two generation Daphnia magna reproduction test and to test the performance of that protocol across up to 20 different labs from Europe, America and Asia. Indeed this is one of the largest conducted intercalibration tests ever conducted using the OECD 2011 guideline. We aimed to develop a two generation test with only few amendments of the already existing guidelines. The few modifications included to start the second generation with third brood neonates collected from reproducing females from the first generation. We selected pyperonil butoxide as a test chemical based on previous studies that showed that this compound was the best candidate among other two compounds. To minimize interlab variability all laboratories used the same stock solutions, which were prepared by one of the partners and send it the rest of partners. The results obtained in this intercalibration test will be discussed in this poster.

WE191

Maternal nanoparticle treatment induces multi-generational effects in the nematode Caenorhabditis elegans

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Organisms produce offspring during the whole of their reproductive life cycle; however, changes in age or environmental conditions may alter physiological characteristics of the parental generation. These changes might result in altered responses to environmental challenges, such as nanoparticles, and might be manifested as phenotypic effects in the offspring. Here, we provide evidence of multi-generational effects following exposure of *Caenorhabditis elegans* to quantum dot (QD) nanoparticles. We identified four groups of F_1 offspring from the treated parental generation based on the timing of their production: first spawning, first appearance of the bag of worms (BOW) phenotype, serious BOW, and lethal BOW. In the F_2 generation derived from the latter group, early reproductive senescence was observed; this effect was associated with abnormal germ cell proliferation during early development. We suggest that RNA interference mechanisms are associated with the lethal BOW phenotype and induce a transgenerational effect that causes reduced F_2 fertility.

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MetaOMICs in ecotoxicology: evaluation of alterations in the structure and functions of ecosystems (P)

WE192

Structural and Functional Biomarkers to assess nanomaterials-associated environmental risk

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Advances in environmental biotechnology have allowed the characterisation of soil organisms, and the measurements of gene and/or protein expression levels upon exposure to chemical stressors, such as engineered nanomaterials (ENMs). The forecasted growth in the manufacture and use of these nanomaterials makes it likely that increases in environmental exposure to ENMs will occur, whereas the toxicological effects of nanomaterials on environmental biota, soil health and functionality remains mostly unknown. A microbial ecotoxicological approach based on the response of soil microbial communities offers the opportunity to evaluate the impact of nanomaterials on natural populations, and it leads consequently to a better environmental risk assesment. Recent innovative breakthroughs in genotypic profiling, ultrafast genome pyrosequencing, metagenomics, metatranscriptomics, metaproteomics and metabolomics along with bioinformatics tools have provided crucial in-sights of microbial communities and their mechanisms to cope with environmental pollutants. Likewise, molecular microbial biomarkers or bioreporters have been developed for in situ detection/ monitoring of environmental pollution. **Structural Biomarkers:** Multiple group-specific rRNA probes targeting prokaryotic and eukaryotic microbial taxa can be used in a **FISH** experiment for simultaneous phylogenetic classification of physiologically active microbial populations in an environmental sample. **Metagenomics** based methods have been useful to determine novel gene families and or microbes involved in bioremediation of xenobiotics. Availability of whole genome sequences from several environmental microorganisms has been useful to determine the gene pool of enzymes involved in the microbial response to anthropogenic pollutants. **Functional Biomarkers: Transcriptomic** or metatranscriptomics tools are used to gain functional in-sights into the activities of environmental microbial communities by studying their mRNA transcriptional profiles. **Proteomics** based investigations have been useful in the identification of key proteins involved in the physiological response of microorganisms when exposed to anthropogenic pollutants (e.g. ENMs). Here we show results of the application of some of these molecular techniques in linking the structure and function relationships of microbial communities and for biological sensing of pollution at ENMs contaminated sites.

WE193

Reprotoxic effects of microcystin-LR and microcystin-producing Microcystis extract on adult medaka fish

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The massive cyanobacterial blooms threaten human health as well as any living organisms in aquatic environment, particularly due to the production of natural toxic components, so-called cyanotoxins. One of the most characterized cyanotoxins are the microcystins (MCs) that can cause a severe hepatotoxicity. In recent years, the potential reproductive toxicity of MCs has been also explored by several laboratories. The chronic exposure to MCs may threaten Human reproductive health via consumption of MCs–contaminated water or fish. In the present work, we investigated the reprotoxic effects of MCs and MC-producing *Microcystis* extract on adult medaka. Medaka were exposed to pure MC -LR 1, 5 $\mu g.L^{-1}$ and MC-producing *Microcystis* extract with 5 $\mu g.L^{-1}$ of equivalent MC-LR for 28 days. Various reproductive parameters including the fecundity, the fertilization success and the egg hatchablity were examined during the exposure, revealing negative effects induced by MC-LR. In addition, sex hormones and vitellogenin levels in plasma were also affected by the exposure. Histological observations indicate that hepatocytes present glycogen storage loss together with cellular damages. Transcriptome profiling of liver was performed using Illumina RNA-seq and around 5M unique reads per library were mapped to the reference genome of medaka. Differential expression analysis identified over 100 disregulated genes from 5 $\mu g.L^{-1}$ MC-LR and MC-producing *Microcystis* extract treatments and gene ontology enrichment analysis reveals that response to stimulus, metabolic process and transcription regulator are highly affected in both females and males. RT-qPCR has been used to investigate the transcriptional level of 10 related genes in gonad. Furthermore, proteomics profiling of liver by using iTRAQ indicates that a set of proteins involved with lipid metabolism, amino acid metabolism, generation of energy and choriogenin/vitellogenin syntheses were statistically significantly different from those of controls. A sex-specific response was implied from both transcriptomics and proteomics profiling. The outcomes obtained through this study will advance our current knowledge on the range and severity of reproductive toxicity and to explore risks on aquatic vertebrate models due to cyanotoxins, that could be further discussed with regard with Human health.

WE194

Microbial community molecular analysis and sequencing linked to mixtures of chemical compounds

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In Europe, the good quality of surface waters is established under the Water Framework Directive (WFD) based either on the chemical monitoring or on the ecological status. There is a growing concern about the combined action of pollutants present in waters. The European Commission is then asking for a stronger effort to ensure that the risks associated with chemical mixtures are better understood and assessed. The correlation between the chemical composition and the microbial population is not straightforward understandable. Tiber River was selected for a pilot study to characterise the microbial profile of three areas differently influenced by anthropogenic pressures (agricultural, urban and industrial) and one pristine area. Water samples were collected from four sampling sites during two seasonal periods (April and October). Tiber River samples were characterized from chemico-physical point of view. Chemical analyses focused on pesticides including biocides and insecticides, benzotriazole, corrosion inhibitors (high production volume chemicals), pharmaceuticals, antibiotics, and perfluoroalkyl substances (e.g. PFOS; PFOA). Chemical analyses revealed low concentrations of pesticides in the agricultural area of Attigliano (sampling point 2). Emerging contaminants (benzotriazoles, pharmaceuticals, perfluoroalkyl substances) were particularly found at the sampling point 3 in the River Aniene in Rome (industrial impact) and sampling point 4 in the Tiber River close to the river mouth, downstream of the urban waste water treatment plant of Southern Rome. In order to link these results to the ecological status of the river, molecular analysis (qPCR-based detection of bacteria, cyanobacteria and protozoans) were also performed in parallel with 16s and shotgun sequencing of the metagenomes of the river microbial community. Concentrated water samples were sequentially filtered using 20 μm, 5 μm and 0.22 μm filter membranes. The microbial community genomic DNAs were extracted from the 0.22 μm filters. A fragment of the 16s rDNA region was amplified and sequenced. To better evaluate bacterial diversity along the river also shotgun sequencing was performed. Based on the results obtained from the 16s and shotgun sequencing, some targeted microorganisms were selected for qPCR assays to find out the potential correlation with the chemical analysis.

WE195

Measuring microbial interactions in soil in high throughput

M. Dubey, University of Lausanne / DMF; J. van der Meer, University of Lausanne / Department of Fundamental Microbiology

Microbial ecosystem engineering approaches often rely on the introduction of one or more selected species into an existing microbial community. The success of introduced species to a large extent depends on the types of interactions that it is developing with other existing microbes, such as neutralism, commensalism, syntrophism or competition. Deciphering the rules governing microbial species' interactions is a strenuous task. Our project is focused on better understanding the principles of success of establishing pure cultures in complex microbial ecosystems such as contained within soil. Here we develop a high-throughput co-cultivation approach that might enable us to study the species' "interactome", the identification of favourable and non-favourable species combinations that decide on the survival of the inoculant in the community. We use agarose micro-beads as growth chambers to randomly combine soil community members with or without pure culture inoculants. Growth of co-cultured species members is followed by microscopy and enables a global overview of potentially positive effects of the inoculant on growth of the members in the soil on specific carbon substrates. In addition, the “interactome” study allows detecting possible species pairs, negative or positive, that can be recovered and identified in detail. The resulting knowledge not only provides ample data in designing functional synthetic communities but also construct new avenues for “synthetic ecology”. \n

WE196

The bactericie triclosan affects 16S and 18S rRNA diversity and composition in marine periphyton communities

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Modern DNA sequencing platforms have an enormous throughput at a lower cost than ever before. The possibility to multiplex and barcode PCR amplified gene fragments, and sequence them in the same run can further lower the cost per sequencing depth. This approach, *amplicon sequencing*, has great potential to detect adverse changes in the structure of toxicant-exposed microbial communities. Here we investigate the long-term effect of the bactericide triclosan (TCS) on the 16S and 18S gene richness, diversity and composition of marine periphyton communities. A long-term (17 days) flow-through microcosm experiment with constant TCS exposures of 0 – 316 nM was performed. DNA was extracted using the FastDNA spin kit (initially developed for for soil communities) and we performed multiplexed amplicon sequencing using the Illumina platform. In total 313,855 16S and 176,566 18S sequences were retrieved and the sequences were clustered into 1789 and 509 Operational Taxonomic Units (OTUs), respectively. The prokaryote part of the communities was dominated by the phyla *Proteobacteria*, *Bacteroidetes* and *Planctomycetes*, whereas the eukaryote part was dominated by *Arthropoda*, *Bacillariophyta*, *Chytridiomycota*, *Cercozoa*, *Mollusca* and *Ciliophora*. TCS exposure resulted in a decrease of 16S richness and diversity, and a changed 16S composition, at an exposure of 31.6 nM and above. In the concentration interval of 1 – 10 nM more subtle changes in the overall 16S composition were detected. For *Proteobacteria* and *Bacteroidetes*, the richnesss and diversity followed a bi-phasic concentration response curve with a clear decrease between 3.16 nM and 31.6 nM, but with a much smaller decrease at 316 nM. This might be an indirect effect from a changed 18S composition at 316 nM. Unfortunately, we do not have 18S data for the exposure interval 10 – 100 nM and hence cannot compare the sensitivity of pro- and eu-karyotes. In general, the 18S composition was more variable than the 16S composition. Still, the TCS exposure resulted in a decrease of the 18S richness and diversity, and a changed 18S composition, at an exposure of 316 nM. Based on richness and diversity, the eukaryotic taxa *Viridiplantae* was sensitive whereas *Stramenopiles* was insensitive to TCS. In this study we demonstrate that triclosan has adverse effects on microbial community composition and that amplicon sequencing is a suitable method to describe toxicant-induced changes in community structure to a reasonable cost.

Challenges in Environmental Assessment of Cosmetics and Personal Care Products (P)

WE197

Importance of Preserving Environmental Risk Assessment (ERA) of Personal Care Products

I. Davies, Personal Care Products Council / Science; A. Carrao, Kao USA / RD

ERA is the process by which exposure and hazard of a material are quantified.

Within such an assessment, environmental occurrence, fate and ecological effects are assessed *via* standard test guidelines, such as those prescribed by the OECD (Organization for Economic Cooperation and Development), or by high quality non-standard experiments. Testing strategies are often tiered, meaning that a number of screening level or modeling methods (such as QSARs, or quantitative structure activity relationships) can be used to prioritize materials for further, more in depth testing. This approach is cost effective and reduces unnecessary animal testing. The result is the generation of a PEC (predicted environmental concentration) and a PNEC (predicted no effects concentration). \n \n Next, a risk characterization ratio (RCR) is derived by dividing the PEC by the PNEC value. The RCR can be either >/< 1 (or in rare cases = 1). If a value of < 1 is calculated, then a material is likely to pose an acceptable environmental risk. Conversely, if an RCR >/= 1, then an unacceptable environmental risk will be posed by the material. \n \n ERA is widely prescribed by a number of major global regulatory bodies and frameworks. These include the USEPA, USFDA, ECHA and VICH. A large number of structurally and functionally diverse materials have therefore been assessed by risk-based methods, or are currently being assessed in this manner. As such, ERA represents a widely used, scientifically relevant and reliable means of determining materials which may be of environmental concern. \n \n Despite the availability and proven utility of ERA methods in identifying materials of potential concern, a number of regulatory and legislative schemes in the US and Europe have abandoned an ERA-based approach when assessing environmental impacts of consumer chemicals. In particular, hazard and exposure data are often considered in isolation. This therefore results in regulatory and legislative decisions being taken without a full comprehension of how a material may impact a specific environment. Moreover, alternative compounds, which are then needed to replace controlled materials, can sometimes pose more of an environmental risk. These are called regrettable substitutions. \n \n This poster will discuss the benefits of ERA compared with only considering hazard or exposure. We will also use examples of regrettable substitutions to highlight instances for which comparative ERAs may have avoided these from occurring.

WE198
PBT Behavior and Aquatic Ecotoxicity of Personal Care Products: QSAR modeling and prioritization
P. Gramatica, S. Cassani, A. Sangion, University of Insubria / DiSTA
Several ingredients in Personal Care Products (PCPs) are now of recognized environmental concern for their increasing presence in aquatic compartments. These ingredients have heterogeneous chemical structures and very different properties, but for the majority of them persistence and toxicity data are lacking. Moreover, the determination of all the dangerous properties, required by REACH and Cosmetics Directive, is a long and difficult task. Due to the high variety of these chemicals and the big number of end-points that should be studied, it is important to have tools useful to highlight the most harmful compounds, focusing the experiments only on these. In this study we propose tools for prioritizing the most dangerous ingredients in PCPs, thus reducing the costs and the animal tests as required by the new Regulations. More than 500 PCPs (fragrances, parabens, phthalates and UV filters) have been screened for their cumulative PBT behavior and for their acute aquatic toxicity on algae, Daphnia and fish. This screening has been done by QSAR models (consensus between PBT-Index in the software QSARINS and US-EPA PBT Profiler) and by *ad hoc* developed QSARs on *P. subcapitata*, *D. magna* and *P. promelas* toxicity. Toxicity predictions were combined by Principal Component Analysis and MultiCriteria Decision Making identifying a cumulative toxicity trend, further modeled by theoretical molecular descriptors as an aquatic toxicity index (ATI). A priority list containing the PCPs identified as the potentially most hazardous, both for PBT behavior and aquatic ecotoxicity, is proposed. The PCP ingredients identified as the most dangerous are mainly UV filters, in particular with benzotriazole rings. The results of this screening/ranking are a valid help to assess the ecological risk of PCP ingredients, belonging to various chemical classes, and to reduce and focus experimental tests.

WE199
Legal gaps in the European chemical legislation impede environmental risk assessment and risk management of personal care products
U. Klaschka, University of Applied Sciences
Some features in the REACH- and the CLP-Regulation seem to be designed especially for products like personal care products with the intention to improve protection of the environment and of human health. However, a closer look reveals several exception provisions with the effect that these rules need not to be applied to cosmetics. Cosmetics are special. But are the exceptions in the European chemical legislation justified? A comparison of the REACH- and the CLP-Regulation with the Cosmetics Regulation reveals that the Cosmetics Regulation cannot compensate for these caps. Several studies show that this situation impedes a decent hazard and risk assessment of personal care products and ingredients.

WE200
Natural substances in personal care products
U. Klaschka, University of Applied Sciences
Natural personal care products enjoy great public acceptance today. Is this bonus justified? Natural substances in personal care products are used in increasing amounts. Most of them are complex mixtures of natural compounds belonging to various chemical substance classes. A large number is classified as dangerous for human health and the environment and many constituents are known for their potent physiological effects. A market study revealed the big variety of natural substances used in personal care products. It also showed that many dangerous natural substances are indeed used today. However, the most frequently employed natural substances are not classified as dangerous, such as Xanthan gum, *Simmondsia chinensis* oil, *Helianthus annuus* seed oil or *Butyrospermum parkii* butter. (Eco-)Toxicity of multi-constituent substances like natural substances is caused by the constituents. Some constituents of the natural substances found are chemically identical to industrially synthesized chemicals.. In cases where the constituents are known, the classification as dangerous for the aquatic environment of the natural substance can be calculated following the procedure of the CLP regulation for mixtures. There is no scientific reason why natural ingredients in personal care products should in general be regarded as less hazardous compared to synthetic ingredients.

WE201
Statistical analysis of siloxanes in biota and sediments in the Inner Oslofjord 2011-2014, with emphasis on D5
M. Schøyen, Norwegian Institute for Water Research (NIVA) / Centre for Coastal and Marine Research; S. Øxnevad, D. Hjermann, Norwegian Institute for Water Research NIVA; C. Mund, T. Böhmer, K. Beckmann, Evonik Nutrition & Care GmbH; D.E. Powell, Dow Corning Corporation / Environmental Sciences
Cyclic volatile methylsiloxanes (cVMS) in biota and sediment of the Inner Oslofjord in Norway have been monitored since 2011 to determine if concentrations are stable or changing following a temporal trend. Cod (*Gadus morhua*), herring (*Clupea harengus*), shrimp (*Pandalus borealis*) and sediment were analysed for octamethylcyclotetrasiloxane (D4), decamethylcyclopentasiloxane (D5) and dodecamethylcyclohexasiloxane (D6). Cod (46-55 cm) were analysed individually (2011, 2012) or as pooled samples (n=3 in 2013, 2014). Herring (Mean concentrations (ng/g lipid) of D4 were greatest in herring (18.4) and cod (17.5), and lowest in shrimp (5.9). For D5 and D6, the concentrations were greatest in herring (respectively 1881 and 26.6), intermediate in cod (respectively 356 and 14.7), and lowest in shrimp (respectively 80 and 2.9). Generally, geometric mean concentrations of cVMS in biota decreased over the first four years and, except for D5, were approaching or were less than the analytical detection limits in the 2014 samples, especially for shrimp. Time trend analysis following OSPAR guidelines (OSPAR 2008) requires five years of data. When applied to the first four years, trend analysis using these guidelines detected no significant trends for D5 in biota or sediment. However, marginally significant decrease (p=0.077) was observed for lipid normalized concentrations of D5 in cod. When using ANOVA, a significant decrease was detected for D4 and D5 in cod, but not for D6. There was high variation in lipid content of cod within and between years. The lipid content of whole cod was reduced by at least 50 percent from 2011 to 2013, and then showed a slight increase in 2014. Thus, a mixed effect model combining whole body lipid content (within years), as well as a between-year effect on lipid-normalized D5 concentrations, demonstrated significant year effect. Similar to that observed for cod, lipid-normalized concentrations of D5 in herring were lowest in 2014, compared to 2011-2013. Herring had higher lipid content and higher concentrations of cVMS than cod. A mixed effect model with whole body lipid fraction versus lipid-normalized D5 concentrations also demonstrated significant year effect for herring. Based on OSPAR guidelines, no significant trend was detected between years for concentration (w.w.) of D5 in the 0-1 cm sediment.

WE202
IFRA ENVIRONMENTAL STANDARDS: RISK AND HAZARD ASSESSMENT UPDATE FOR 2016
A. Lapczynski, RIFM / Environmental Specialist; D.T. Salvito, Research Institute for Fragrance Materials (RIFM) / Dept of Environmental Science; M. Vey, C. González, IFRA
To assure safety of fragrance ingredients in consumer products, International Fragrance Association expanded the fragrance industry's self-regulatory safety program with the development of IFRA Environmental Standards for both risk and hazard in 2008. Fragrance material risk assessments for these Standards are incorporated in the Research Institute for Fragrance Materials' (RIFM) testing program in coordination with its Expert Panel. To identify materials for risk assessment refinement, fragrance materials were screened using the RIFM Environmental framework and 2008 IFRA volume of use survey as reported for both Europe and North America. The Framework for this evaluation was published in Environment Toxicology and Chemistry (Salvito et al., 2002, 1301-1308). In addition, hazard assessment on these materials was also performed and reviewed. As a result nearly 3,000 materials were screened with preliminary risk quotients estimated to rank priority materials for risk assessment refinement. In an effort to provide greater transparency to the IFRA Environmental Standards, RIFM reports the most recent results of these additional tests (for both risk and hazard assessments) at both the annual SETAC NA and Europe meetings. These studies include persistence testing (ready biodegradation tests and die-away

studies), bioaccumulation, and acute and chronic aquatic toxicity. Incorporating these new data in a second tier risk and hazard assessment for these materials will also be presented.

WE203

Comprehensive review of several surfactants in the marine environment: fate and toxicity

M. Jackson, Shell Health / Shell Health Risk Science Team; C.V. Eadsforth, Shell International / Shell Health; D. Schowanek, Procter & Gamble / Brussels Innovation Center; T. Delfosse, The Procter & Gamble Company / Product Safety Regulatory Affairs; A. Riddle, Ecospan; N. Budgen, AstraZeneca

Numerous environmental risk assessments of surfactants in the freshwater compartment have been developed (HPV, i.e. HERA, OECD REACh, etc), which can serve as a basis for the marine compartment through extrapolation of freshwater data to marine data. However, risk assessment of surfactants in the marine environment, based upon experimental marine data has hardly been investigated. To investigate the extent and quality of available data from marine studies (water and sediment), ERASM (Environmental Risk ASsessment and Management) commissioned a compilation of marine exposure and effects data for a number of current and historical surfactants (alcohol ethoxylates (AE), alkyl sulphates (AS), alcohol ethoxysulphates (AES), linear alkylbenzene sulfonates (LAS), and ditallow dimethyl ammonium chloride (DTDMAC)). Industry sources and the literature identified, evaluated and reported a variety of biodegradation and ecotoxicological studies. Marine biodegradation studies reported half lives in the range of 2-28 days (AE), 0.3-20 days (AS), 1-50 days (AES), and 0.3-45 days (LAS) (no biodegradation studies were found for DTDMAC). A wide variation in the number of published marine ecotoxicological studies was found for each of the surfactant classes. AS and LAS have been studied most, and AE, AES and DTDMAC the least. Marine invertebrates are the most studied class of organism in both acute and chronic toxicological assessments, whereas plants and fish are comparatively much less studied, particularly in terms of chronic assessments. Based on data ranges and mean toxicities from the studies reviewed, it was concluded that the toxicity of the five surfactants, from highest to lowest is: LAS > AS > AE > DTDMAC > AES. Acute toxicity (for algae, invertebrates and fish) was shown to be generally equisensitive across the board. Where it was possible to compare marine and freshwater ecotoxicological values, marine data ranges generally fell within typical freshwater data ranges. Marine bioconcentration data are virtually non-existent, and were only available for LAS and AS. Significant gaps in data were found in the marine ecotoxicology assessments for all surfactant classes.

WE204

Fate and effect of sediment-associated triclosan in a freshwater microcosm setup: the relative importance of bioaccumulation and biotransformation

R. Windfeld, Roskilde University / ENSPAC; I. Roessink, Alterra / ERA team; P. van den Brink, Alterra and Wageningen University / Aquatic Ecology and Water Quality Management Group; D.T. Salvito, Research Institute for Fragrance Materials (RIFM) / Dept of Environmental Science; H. Selck, Roskilde University / Dept Environmental Social and Spatial Change

Aquatic environments are increasingly exposed to household and personal care products (HPCs) from their extensive use in urbanized areas. The environmental fate of these chemicals and their impact on benthic communities in receiving waters is not completely understood. This study aims to examine the fate of sediment-associated triclosan (TCS) as well as its toxic impact on the benthic community in a fresh water microcosm system. To investigate toxicity of TCS, a community of benthic invertebrates was exposed for 60 days to a high and a low concentration of TCS. The community consisted of a range of organisms; *Gammarus pulex, Tubifex tubifex, Lumbriculus variegatus, Asellus aquaticus* and *Caenis horaria*. The impact on the community was recorded as decreasing organism abundance. Benthic systems containing *T. tubifex* and *G. pulex* were employed to examine the fate of TCS during 30 and 60 days, respectively. The biotransformation capacity of these organisms was assessed by measuring the concentration of TCS and selected metabolites in overlying water, sediment and organism tissue after the two exposure intervals, respectively. The results will be discussed in the context of how bioaccumulation and biotransformation of the products will affect the environmental fate of these compounds

WE205

LogP: a classic endpoint for ERA, but what happens when the main part of the test substance is a resin-like insoluble, volatile, mostly solid, complex mixture and constituents are unknown?

B. CASENAVE, CEHTRA SAS; S. LEGAY, FCBA; R. SAMSERA, CEHTRA SAS; P. Thomas, CEHTRA SAS / Ecotoxicology and Risk Assessment

Under the REACH review program several kinds of substances are considered: monoconstituents, multiconstituents & UVCBs. Across these substance types several families always present a challenge to test especially in physico-chemistry, ecotoxicology and environment. One of these challenging groups is fragrances. \n Fragrance chemicals fall under multiple categories, they can be natural, synthetic, monoconstituent, multiconstituent or considered as UVCBs. One group of fragrances that falls under the title of multiconstituent/UVCB are known to be most difficult to assess: Essential oils. Essential oils (EO) are complex mixtures, with differing but often low solubility, high volatility and are known to contain some toxic constituents such as terpenes. Some of these substances are even more complex: gums, resinoids and concretes, extract from natural raw material like essential oils. They are as complex as EO but their composition is mostly unknown and their physical state leads to further difficulties for physico-chemical and ecotoxicity testing: they are (mostly) solid(ish), extremely viscous resins, with a frozen honey-like texture. However, some of these substances still require environmental risk assessment under the registration process. Therefore some key PhysicoChemical endpoints are needed, such as water solubility and/or LogP values. It is difficult to determine the LogP value of a substance which is solid(ish) with a composition which is basically unknown except for the fact that the number of constituents amounts to potentially well over a hundred. We discuss the protocol we designed to meet the requirements of the log Kow endpoint following the OECD 123 (Slow Stir & HPLC) guideline for one of these substances and how we expect to use this result in the REACH Chemical Safety Report. Keywords : risk, assessment, logP, fragrance

WE206

Ecotoxicity of natural complex mixtures: raw material of plant extracts which are resin-like solids.

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Under REACH review program several kinds of substances are considered: monoconstituents, multiconstituents, & UVCBs. Across these substance types several families always present a challenge to test especially in ecotoxicology and environment. One of these challenging groups is fragrances. \n Fragrance chemicals fall under multiple categories, they can be natural, synthetic, monoconstituent, multiconstituent or considered as UVCBs. One group of fragrances that falls under the title of multiconstituent/UVCB are known to be most difficult to assess: Essential oils. Essential oils (EO) are complex mixtures, with differing but often low solubility, high volatility and are known to contain some toxic constituents such as terpenes. Some of these substances are even more complex: gums, resinoids and concretes, sub-categories of essential oils. They are as complex as EO but their composition is mostly unknown and their physical state leads to further difficulties for ecotoxicity testing: they are (mostly) solid(ish), extremely viscous resins, with a frozen honey-like texture. In order to acquire the data required for registration of these substances, we performed a series of OECD 202 (daphnid acute studies) using WAF methodology, with a protocol adapted to fit the substances in question, taking into account the particular properties of these substances in an attempt to keep the results robust from a regulatory point of view. We present and discuss these results on their ecotoxicity properties and the conclusion that we can draw from such results. Keywords: fragrance, raw, material, ecotoxicity

WE207

A thermodynamically-based step by step calculation method to assess aquatic toxicity of mixtures

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From an environmental perspective chemical substances do not act in the same way as pure substances tested in the lab. Many substances, classified as such under REACH are actually mixtures (i.e. Natural Complex Substances, such as essential oils). Therefore this is a need to understand mixture toxicity and since water is the universal solvent in the environment, aquatic toxicity of mixtures has been of high concern for many years. One recognised way to obtain toxicity data on mixtures was to follow OECD (2000) Guidance No. 23 on difficult substances and mixtures which advocates use of the water-accommodated fraction (WAF) method (i.e. testing the constituents together at specific loading concentrations). Implementation of the WAF method costs time and money. More recently, research has been conducted in order to determine if an additivity approach can be used to predict aquatic toxicity of mixtures of chemicals or whether independent action predominates. Nowadays the favoured hypothesis is the Concentration Addition (CA) where toxic units of mixture constituents with same toxic mode of action can be summed (Loewe and Muischnek, 1926; Backhaus *et al*., 2003). In this work, the chemical activity concept has been used to normalise the relative part of toxicity of each constituent (Mackay *et al*., 2009; Thomas *et al*., 2015). Schmidt *et al*. (2013) and Smith *et al*. (2013) have both shown that aquatic and terrestrial ecotoxicity can be determined by using summing constituent activities of sub-lethal levels of constituents in a mixture comprised of solid PAHs. However unlike solids compounds (Banerjee *et al*., 1984), liquids partition into each other thus reducing the overall toxicity of the mixture. Therefore the initial rule of CA modified according to the chemical activity has also to be completed by an additional step where the non-bioavailability phase is remove from the calculation. Based on thermodynamic liquid-liquid equilibrium, aquatic toxicity can finally be accurately calculated for mixtures of liquid hydrophobic compounds, like essential oils. This method can provide results within a factor of 2 of experimental results while the CLP additivity method results are at least a factor of 10 lower.

WE208

Predictive laboratory methodology to assess coral bleaching: application to UV filters

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Increasing ocean temperature and acidification, overfishing, coastal development and pollution are well known stressors on coral reefs. They may induce coral bleaching, a process by which corals lose their symbiotic microalgae (zooxanthellae). Ultimately, corals may die when these stressful environmental conditions last too long. Weakened corals, more susceptible to infectious diseases, show poor resilience from episodic bleaching events. Some studies have reported that certain UV filters (mostly 4-methylbenzylidene-camphor, benzophenones and octylmethoxycinnamate) contained in sunscreens lotions and washed off by swimmers, could contribute to coral bleaching. Media took it for granted and suspicion has been extended to all organic UV filters present in sunscreens products. The present study was aimed at clarifying the potential effect that organic UV filters (such as Avobenzone, Octocrylene, Terephtalylidene-dicamphor sulfonic acid, Silatrizole, etc…) may have on different coral species. Two herbicides (Monuron and Diuron) were used as positive references. First a preliminary laboratory screening test was developed to assess potential adverse effect of short exposure (48h) to elevated concentrations (from 1 to 100 mg/L) of the compounds. As a sublethal endpoint predictive of coral bleaching, chlorophyll photosynthetic efficiency of the symbiotic micro-algae (zooxanthellae) was monitored with PAM (Pulse Amplitude Modulated) fluorimetry on nubbins of hard coral species *Seriatopora caliendrum* and *Styllophora pistillata* In a second step, coral nubbins of *Styllophora pistillata* (hard coral) and *Turbinaria reniformis* (soft coral) were exposed for 5 weeks at lower concentrations in 15 liters aquariums, under semi static conditions with weekly solution renewal. A specific analytical methodology was developed, combining automated solid phase extraction with UPLC-UV detection, to monitor the compounds concentrations in sea water and analyze large number of samples. 5 weeks of chronic exposure to these UV filters at concentrations above those reported in natural sea waters, did not induce coral bleaching nor reduce the photosynthetic efficiency of the symbiotic micro-algae.

WE209

iSTREEM 2.0: new enhancements for down-the-drain modeling to support environmental aquatic exposure assessments for cosmetics and personal care products

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The iSTREEM® model ("in-stream exposure model"), a free and publically-available web-based model supported by the American Cleaning Institute (istreem.org), provides a means to estimate chemical concentrations in effluent, receiving waters, and drinking water intakes (DWI) across the conterminous U.S. as well a number of watersheds in Canada under mean annual and low-flow (7Q10) conditions. This presentation will discuss recent upgrades made to enhance the model, underlying data, algorithms and presentation of results in the new version. iSTREEM® 2.0 incorporates geographic locations of over 12,000 wastewater treatment plant (WWTP) facilities along more than 300,000 segments of effluent-impacted river reaches, providing a framework to integrate geographic data to assess environmental risk for multiple scenarios of interest. WWTP facilities and associated facility level information were derived from the latest available USEPA Clean Watershed Needs Surveys. The river network used by iSTREEM® 2.0 was upgraded to a higher-resolution hydrologic dataset based on the USGS/USEPA NHDPlus version 2, which includes estimated mean annual and low flow (7Q10) data based on USGS stream gage measurements. Model results are presented in a standardized manner for consistent results communication across all users and are provided in a readily usable format (MS Excel) for easy interpretation and further customization of result presentation. Major assumptions used in constructing the model will be discussed. Recent developments are geared to expand adoption of the model by a wide variety of users as an environmental risk assessment tool across multiple commodity groups (cosmetics, personal care products, pharmaceuticals, food additives, pesticides, etc.) that require internal or regulatory environmental assessments. The discussion will also include a comparison of model results between the prior version of iSTREEM® and latest iSTREEM® 2.0 to examine the impact of recent upgrades on the national distribution of predicted environmental concentrations.

Endocrine Disruptors: Exposure, Hazard & Risk Assessment (P)

WE210

Is nitrate an endocrine active compound in fish?

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Nitrate and nitrite taken up into fish may be reduced to NO which is known to be a signalling compound in the organism contributing to the regulation of i.e. steroid synthesis. Exposure of male rats to nitrate and nitrite results in reduced plasma concentrations of testosterone (also nitrate concentrations around or below the limits for drinking water). Nitrate concentrations in streams may be elevated due to releases from agricultural practices. The effects of nitrate and nitrite on endocrine relevant endpoints were investigated in zebrafish (*Danio rerio*) and brown trout (*Salmo trutta*). Zebrafish were exposed to nitrate and nitrite from hatch to sexual maturation (60 d) and sex ratio and vitellogenin concentrations were determined. Juvenile brown trout were exposed in a short-term experiment and the concentrations of vitellogenin were determined. The sex ratio in zebrafish was not affected by exposure to environmentally realistic concentrations of nitrate and nitrite but vitellogenin concentrations were slightly elevated in all of the exposed groups compared to the control; no obvious concentration-response relationship was observed. The outcome of the brown trout experiment is awaiting the final analyses.

WE211

Neuro-endocrine disruption in molluscs - Effects of pharmaceuticals on a serotonin- and dopamine dependent system

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The Mollusca phylum is the second largest animal phylum with around 85,000 registered mollusc species and increasing attention to effects of chemicals on the molluscan endocrine system have been given during the last years. This includes initiation of the development of OECD test guidelines (TG) to assess the effect of chemicals in molluscs. To date no endocrine specific mollusc biomarkers have though been validated and included in draft test guidelines due to lack of knowledge of the endocrine system. Here we investigate effects of pharmaceuticals targeting serotonin and dopamine in a cost efficient and fast *in vivo* system using embryos of the freshwater pulmonate gastropod *Lymnaea stagnalis* (the great pond snail). It is known that serotonin and dopamine are involved in many reproductive processes in molluscs Incl. egg maturation and spawning and that pedal ciliary activity causing *L. stagnalis* embryos to rotate in the eggs is serotonin/dopamine regulated. We have developed a system to quantify embryo rotation and present results of exposure to serotonin, dopamine and different anti-depressive pharmaceuticals (e.g. selective serotonin reuptake inhibitors, SSRI´s) using the *L. stagnalis* embryo rotation test system.

WE212

In silico Screening for Potential Endocrine Disruptors

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Endocrine disrupting activities of chemicals trigger adverse effects on organisms. Endocrine disrupters are suspected to be responsible for a number of diseases as testis and breast cancer, oligospermia of young men, or adiposis. Compounds with endocrine activity are labelled as substances of very high concern (SVHC) and are subject of regulations as e.g. under REACH. However, there are so far no internationally harmonised criteria to identify endocrine active chemicals. Furthermore, the endocrine system is rather diverse. Despite of several already developed OECD tests to reveal respective activities, it is still not clear whether they comprise all (eco)toxicologically relevant pathways. Even more, these tests are rather expensive with respect to costs and duration. Consequently, the number of existing data is limited. In silico tools are promising alternatives to avoid testing or at least to allow for the prioritisation of tests by screening larger compound lists. The German Federal Environment Agency holds a prototype of a structural alert model and corresponding software tool for this task. Based on it, the current study aims to significantly extend this model and to make it available as an automated tool in the software system ChemProp (UFZ Department of Ecological Chemistry 2015. ChemProp 6.3. http://www.ufz.de/index.php?en=6738). The envisaged model will provide a decision tree to identify potential endocrine disruptors and their respective modes of action. The paper gives an overview of the approach and reports results for several modes of action with respect to thyroid hormones. Structural rules to identify potential agonists and antagonists for effects on the ligand bonding domain (LBD) as well as for potential antagonists against the activation factor 2 (AF-2) have been established. Furthermore, the opportunity to derive specific rules for enzyme/protein interaction, thyroid receptor interaction, aryl-hydrocarbon-receptor interaction, and other modes of action based on existing data and mechanistic knowledge has been explored. *Acknowledgment:* This study is financially supported by the German Federal Environment Agency, FKZ 3714 63 412 0.

WE213

Evaluation of anthropogenic micropollutants in drinking water: the Tox-Box approach

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Anthropogenic micropollutants in drinking water have been a concern for several years now. Due to analytical improvements more substances are found in drinking water and their presence has to be evaluated to provide the public with save drinking water. So far only lengthy test protocols mostly in animals are used. The aim of the joint project Tox-Box is therefore the establishment of a test battery that allows for the evaluation of a substance in terms of possible genotoxicity, neurotoxicity and endocrine disruptive effects using in vitro approaches wherever applicable. Based on the theoretical concept of the Health Related Indicator Value (HRIV), published by the German Federal Environment Agency (Umweltbundesamt, UBA) in 2003 a practical approach was designed. For each toxicological endpoint several tests were chosen, which provide for a fast and reliable risk assessment, once a chemical is found. The test battery for genotoxicity will encompass the Umu test, in addition to Ames test and micronucleus test. The assessment of neurotoxicity will consist of testing for necrosis, apoptosis and oxidative stress by comparing neural cells with liver cells as well as assays specific for neurons like neurite outgrowth and MAP kinases. Moreover, the activity of acetyl choline esterase and the development of lateral line organs in zebra fish (*Danio rerio*) will be tested. A third set of tests deals with endocrine effects. These are assessed by applying hormone specific reporter gene assays in combination with the H295R assay. When one of these tests is positive, a reproduction assay in *Potamopyrgus antipodarum* snails will be performed for further clarification. During the project some 60 chemicals were tested by the different project partners. The test scheme which was developed suggests that it will meet the demands for fast and reliable assessment. It will also be in line with the Tox21 program which aims for an increase in *in vitro* testing. From a regulatory perspective, genotoxicity is considered the most relevant endpoint. Since genotoxic effects currently result in the lowest cut-off value, no further testing is required, once a substance is considered genotoxic. When no genotoxic effects can be found the assays for neurotoxic and endocrine effects will be carried out. The guideline for testing will be published as a handbook, first in German. An English version is planned for the near future.

WE214

Investigation of (anti)estrogenicity of triclosan using in vitro and in vivo zebrafish bioassays

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Triclosan (TCS) is a wide spectrum antimicrobial agent used in many personal care products (PCPs) that has been detected in surface water and biota. TCS is suspected to be an endocrine disrupting chemical (EDC) and to interact with the estrogen and androgen signaling pathways. However, data available regarding its estrogenic or anti-estrogenic potency provide contradictory information, highlighting the uncertainty regarding its endocrine disruptive potential. The few studies published so far on chronic reproductive effects of TCS on fish report ambiguous results, i.e. a male bias sex ratio in Japanese medaka but a vitellogenin induction in male mosquito fish. In this context, our study aimed to investigate whether TCS acts as an agonist or an antagonist of the estrogen receptor (ER) in human (*in vitro*) and in zebrafish (*in vitro* and *in vivo*). For this purpose, we assessed luciferase-dependent ER transactivation *in vitro* using a human breast cancer reporter cell line expressing hERα (MELN cells), and three zebrafish (zf) liver cell lines (ZELH) stably transfected with zfER (i.e. ZELH-zfERα, -zfERß1 and -zfERß2). In addition, *cyp19a1b* gene induction, which is strictly ER-regulated, was assessed in transgenic *cyp19a1b-GFP* zebrafish embryos (EASZY assay). Our results showed that TCS alone from 3nM to 10μM was unable to induce luciferase activity whatever the *in vitro* models used. At higher concentrations, cytotoxic effects were observed. Co-exposure of cells with E2 0.1nM (ZELH-zfERß1, ZELH-zfERß2 and MELN) or E2 3nM (ZELH-zfERα) and increasing concentrations of TCS had no effect on the E2-induced luciferase activity in either human or zebrafish cells, suggesting that TCS did not elicit any antagonist activity. *In vivo*, TCS alone did not induce GFP in transgenic zebrafish embryos, but decreased the basal *cyp19a1b* expression at 1μM. This effect could reflect the developmental toxicity of TCS as revealed by lethargy and swimming alteration observed in exposed-fish at 1μM. Co-exposure with TCS 1μM and E2 resulted in ambiguous responses: TCS 1μM decreased E2-induced *cyp19a1b* expression by 24% as compared to E2 10nM alone, whereas it slightly increased E2-induced *cyp19a1b* expression at 0.625 nM. All together, our data indicate that TCS does not alter the ER signaling *in vitro* in either human and zebrafish cell lines but seems to alter the E2-induced response *in vivo* through unknown mechanisms.

WE215

Development of a zebrafish embryo test for environmental risk assessment of pharmaceuticals with endocrine disrupting properties

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Pharmaceutical companies are obligated to perform an environmental risk assessment for each new drug that is launched on the market. The mandatory tests for potential endocrine disrupting (ED) compounds require a lot of test animals, which is not consistent with the 3R principle. Therefore, the aim of this study is to develop a zebrafish embryo test, which is not considered an animal test according to the European regulation on the use of laboratory animals, and which is capable of detecting and discriminating among 5 endocrine disrupting modes of action (MOAs). The MOAs that will be studied are aromatase inhibition, estrogen receptor (ER) agonism and antagonism, androgen receptor (AR) agonism and antagonism. As a first stage of test development, 1 pharmaceutical per MOA was investigated to map differences among the 5 compounds in zebrafish embryos at the morphological, physiological and molecular level. We are currently exposing transgenic zebrafish embryos of 2 lines to aid in discriminating among the MOAs: *SR4G* (fluorescent signal after glucocorticoid receptor activation) and *5xERE:GFP* (reports estrogen receptor activation). An ontogeny study is ongoing to determine the gene transcription levels of the receptors, biomarkers and enzymes of the steriodogenesis pathway which will be compared to the gene transcription levels of embryos which were exposed to each of the 5 pharmaceuticals. As a model for ER agonism, 17α-ethinylestradiol (EE2) was used. Fulvestrant was used to investigate ER antagonism. 17β-trenbolone and flutamide were respectively the AR agonist and antagonist. Letrozole was used as a model for aromatase inhibition. Both receptor agonists showed some sublethal effects, while no sublethal effects were observed after exposure to flutamide. After EE2 exposure, the embryos showed sublethal, dose-dependent skeletal malformations of the spine. Cranial malformations were only significant around the LC_{50} concentration and above. For 17β-trenbolone heart rate and growth were already significantly decreased at sublethal concentrations. Swim bladder inflation and swimming behaviour were impaired for both agonists at sublethal concentrations. The data available from 5 medicines with 5 different ED MOAs show the potential of a combination of different datatypes to distinguish among the 5 MOAs. In order to establish a consistent profile that can be used for ED screening of medicines, additional compounds will be tested.

WE216

CYTOCHROME P450 AS POTENTIAL TARGET FOR ENDOCRINE DISRUPTION IN TILAPIAS CAGED IN FOUR RESERVOIRS OF IGUAÇU RIVER

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Environmental chemicals originating from human activities may enter aquatic environments and interfere with the endocrine system of biota. The aim of this work was to investigate endocrine effects in male tilapia (*Oreochromis niloticus*) caged in four cascating reservoirs of the Iguaçu River. RT-qPCR analysis revealed different transcript levels of cytochrome P450s (CYPs) in liver, brain and gonads between the four groups and control fish maintained in the laboratory. Expression of *cyp1a* and *cyp19* aromatase in liver and brain, respectively, was higher in tilapias from the Salto Caxias (SC) reservoir than laboratory controls, but *cyp3a* in the gonads of these individuals was downregulated. In tilapia from Salto Santiago (SS) reservoir, *cyp3a* in liver and *cyp19* in gonads was up and downregulated, respectively. Despite no differences in vitellogenin expression and CYP19 activity, CYP3A activity (testosterone hydroxylase) in liver was inhibited in tilapia from all four reservoirs when compared to the control group. The results obtained in this study indicate that CYPs 450 represents a potential target for chemicals available in the four reservoirs. Since CYPs have many roles, such as biotransformation of xenobiotics and steroids, normal biosynthesis and clearance may be compromised in fish exposed to the water from Iguaçu River.

WE217

Butyltin levels and imposex occurrence in Hexaplex trunculus (Gastropoda, Muricidae) from the Northern Adriatic Sea (Italy) after the EU ban

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Imposex (the development of additional male sex organs in females Caenogastropods) is an endocrine disruption effect commonly used to indirectly assess the presence of tributyltin (TBT), a compound used worldwide in industrial and agricultural applications, mostly in antifouling paints, and now ubiquitous in the marine and transitional environments. Organotins were banned in the

European Union by the EC Regulation 782/2003 and TBT compounds are among the priority hazardous substances according to the European Water Framework Directive 2000/60/EC and its daughter Directive 2008/105/EC. This study aims to assess the butyltin (BT) content in *Hexaplex trunculus* from the Venice Lagoon and the Northern Adriatic Sea after the EU ban. In 2013-2015, gastropods were collected in five stations (2 in the Venice Lagoon, 3 in the Adriatic Sea) in order to assess their BT (TBT, DBT, MBT) contamination and imposex levels. The stations were chosen among those previously surveyed in the same area, for a temporal comparison. Very low BT concentrations (range ΣBT: 16-29 ng cation/g d.w.) and imposex levels (range %I: 5-38%; VDSI < 3.3; RPSI < 0.10%) were found, with a clear decline respect to previous studies in the same area. Quite similar values were shown in lagoon and off-shore samples. TBT levels and VDSI values determined in this study are consistent with those reported in literature for the same species from other geographical areas, with VDS< 3 at TBT concentrations of 15 ng/g d.w.. However, contrary to previous surveys in the Venice Lagoon, imposex indices and female penis lengths did not correlate with BT body burden in females. This finding could be explained by the high sensitiveness of the species that is known to show an initiation of imposex at concentrations lower than 1 ng Sn/g d.w. (2.44 ngTBT/g). Moreover, also the development of imposex exhibited a different course respect to that observed before TBT ban, when BT concentrations and imposex levels were higher. Previous studies documented first the development of the incipient penis, followed by the development of vas deferens. In this study, the onset of imposex occurred first with the appearance of the vas deferens and then with the development of the incipient penis. In this regard, it cannot be excluded that other factors may alter the endocrine control of imposex development, such as exposure to heavy metals, changes in environmental conditions and the influence of other endocrine disrupting chemicals.

WE218
An integrative study design for a zebrafish full life cycle toxicity test
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Fish full life cycle (FFLC) toxicity tests are considered to provide definitive assessments for population relevant endpoints, including those mediated by endocrine mechanisms (i.e. OECD Conceptual Framework level 5). These tests have been conducted with various fish species possessing diverse life history traits, often as part of a global registration package for plant protection product active substances. A life cycle test protocol with limited validation (OECD TG 240) exists for the medaka (*Oryzias latipes*), but other species such as the zebrafish (*Danio rerio*) are more commonly used for toxicity testing and research. In recent years, several fish test guidelines have been released, which are targeted to screen for endocrine effects on specific stages of the fish life cycle and have been robustly validated using zebrafish. Herein we describe a study design for a zebrafish full life cycle toxicity test that integrates critical aspects from previously validated guidelines. The exposure starts with freshly fertilized eggs (F0-generation) from a non-exposed population. The effect of the test substance on the survival, growth, and sexual development of the F0-generation are evaluated (consistent with OECD TGs 210 and 234). After the onset of consistent spawning behavior (approx. 70 days post hatching), reproduction is assessed as fecundity and fertility (consistent with OECD TG 229). Upon sacrifice, the phenotypic sex of adult F0 fish is confirmed and biomarkers such as vitellogenin can be measured (consistent with OECD TG 229 and 234). Fertilized eggs produced by the F0 test groups are used to start the F1-generation, which is exposed for about 34 days (until approximately 30 days post hatch), to evaluate effects on hatchability, development, survival and growth of the F1 early life stages (consistent with OECD TG 210). We present data demonstrating successful zebrafish growth, sexual development and reproductive performance and provide a statistical power analysis for apical population relevant endpoints.

WE219
A multi-generation study on the effects of 17β-dihydroequilin, E1 and E2 in the medaka (Oryzias latipes) following a combined protocol based on the OECD 229 and OECD 234 Guidelines
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Two separate studies were conducted to understand the endocrine effects of 17β-estradiol and Conjugated Estrogens (CE), specifically, estrone (E1) and dihydroequilin (DHE) on three generations of Japanese medaka. In the multigeneration life-cycle test, medaka were exposed to nominal concentrations of 1.0, 7.5 and 75 ng a.i./L each of E1 and DHE, for total exposure of 2.0, 15 and 150 ng a.i./L in combination, a positive control of 100 ng a.i./L E2 and a negative control. Two additional dosing groups were exposed; one to 1.0 ng a.i./L E1 alone and the other to 1.0 ng a.i./L DHE alone. Following a pre-exposure period of 26 days to identify actively spawning fish, spawning groups of 5 males and 5 females were formed and exposed for 21 days under flow-through conditions to evaluate reproduction of the parental generation (F0) as per OECD 229, Fish Short Term Reproduction Assay (FSTRA). Following the FSTRA, an evaluation of sexual development in the F1 generation using eggs from the F0 generation carried through 83 days post hatch (dph) was conducted as per OECD 234, Fish Sexual Development Test (FSDT). Following the 83 day exposure of the F1 generation, most of the treatment groups were terminated in keeping with the FSDT. However, exposures in the 2.0 and 15.0 ng a.i./L E1/DHE combination doses, as well as the negative control were continued through reproduction (22 days) and hatching of the F2 generation embryos (4 dph). A similar study was conducted to evaluate 17β- estradiol at nominal concentrations of 2.75, 8.8, 28.2 and 90.1 ng/L. Survival, growth, reproduction (fecundity and fertility), hatching success, sex ratio and VTG data will be presented for these three endocrine disrupting compounds.

WE220
Is the fungicide tebuconazole toxic for the reproduction of aquatic invertebrates?
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The triazole fungicide tebuconazole acts via an inhibition of the ergosterol synthesis of fungi, but it is also suspected to interfere with the steroidogenesis pathway. In rats, tebuconazole impairs reproduction by an interference with the endocrine system. Whether tebuconazole shows similar effects in invertebrates is unknown, even though for some invertebrate species effects of endocrine disrupting chemicals have been described. As tebuconazole occurs in surface waters at concentrations up to 590 ng/L (Hesse, Germany), the fungicide may interfere with the reproduction of aquatic invertebrates and therefore may pose a risk for invertebrate populations. \nIn order to investigate the toxicity of tebuconazole, long-term reproduction tests with the snail *Potamopyrgus antipodarum* (28 days) and the water flea *Daphnia magna* (21 days) were conducted. The number of embryos and the number of offspring served as endpoints for *P. antipodarum* and *D. magna*, respectively. In addition, the formation of male offspring was investigated in *D. magna* as an indicator for endocrine disruption. Other pesticides, mostly insecticides known as juvenile hormone analogs have shown to induce male formation. \nThe results of the ongoing study will be presented and discussed. \nAcknowledgments: This study is funded by the Federal Ministry of Education and Research (BMBF, Germany) within the framework of the project “NiddaMan” (02WRM1367A).

WE221
A preliminary assessment of human health risk due to inhalation of atmospheric POPs in an agricultural area in Turkey
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Persistent organic pollutants are a group of chemicals that resist to degradation in the environment, bioaccumulative, have toxic effects and undergo long range atmospheric/aquatic transport. Among these chemicals, there exist commercially produced halogenated chemicals such as polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs). These chemicals are of public concern due to their adverse health effects both on humans and wildlife even at very low concentrations. Similar to other regions of the world, OCPs were widely used for agricultural purposes in Turkey. Additionally, even though never produced in Turkey, PCBs were imported for various applications. As the atmospheric transport is main mechanism in the cycling of these chemicals, inhalation is one of the important exposure pathways and health risk assessment is recommended. In this study, atmospheric OCPs and PCBs concentrations were measured by passive sampling in an agricultural region. In addition, the cancer risks for lifetime inhalation of OCPs and PCBs were calculated based on these atmospheric levels. Total OCPs and PCBs concentrations were 266-2374 $pg.m^{-3}$ and 1.40-283 $pg.m^{-3}$, respectively. But one sampling site, the total lifetime cancer risks due to inhalation of OCPs and PCBs were lower than the acceptable risk level of 1×10^{-6} at all sites. Calculations showed that children exposure is about two times higher than adult exposure. Since there are three main exposure pathways namely ingestion, inhalation and dermal absorption, other exposure routes should also be evaluated in order to assess the total risk.

WE222
Air multi-contamination by endocrine disruptors in urban area of Paris (France): characterization by chemical and biological analyses
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The continuous contamination of atmosphere by a number of endocrine-disrupting (ED) compounds involves new societal concerns about the importance of human exposure to low dose of compounds in mixture and subsequent effects upon health. Up to now, that question was poorly documented and knowledge improvements are needed about ED compounds transfers in ambient air, both outdoors and indoors, and also, towards humans. In parallel, hazards arising from their intake by the respiratory tract (*via* gaseous or particulate phases) need to be specified. In this context, the multi-contamination of both the two atmospheric phases, gaseous and particulate, was characterized in urban area of Paris (France) by a two-track approach: the determination of the air contamination levels in several ED compounds families (plasticizers, surfactants, dielectrics, flame retardants, and compounds from combustion synthesis), and the evaluation of the

ED potency of the "cocktail" of air contaminants on bioassays integrating interactive effects between compounds. Disruptions of the transcriptional activity depending on nuclear receptors to estrogens, androgens and thyroid hormones were thus studied, since alterations of these endocrine systems were previously reported in animals and humans. In this way, the air contamination from an urban area was compared during contrasted seasons with this from a forest environment and a suburban area, or with this from indoor living places including three different urban habitats (apartment, office, day nursery). All the ED compounds appeared ubiquitous, as well outdoors as indoors. Except PAHs, they showed indoor concentrations 10 to 80 times higher than the indoor ones. In addition, contrary to *a priori* ideas based upon the low vapour pressures of semi-organic volatile compounds, it appears that human exposure *via* the respiratory tract occurs mainly through the gaseous phase, which is supported by the international literature. Theses contamination data associated with the known ED nature of the target compound were in accordance with the relative ED potency of the ambient air between atmospheric phases, seasons or environments, and the nature of the highlighted ED effects: estrogenic, anti-androgenic and thyroid-like. The chronic human exposure to indoor air contamination, namely *via* the gaseous phase, might induce adverse sanitary events related to the ED potency of that contamination.

WE223

Estimating the carcinogenic and non-carcinogenic effects of persistent organic pollutants in food stuffs using risk assessment indices

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Chemicals play a part in almost all human activities including medicine and agriculture. However, there is a staggering mismanagement of chemicals in Nigeria, leading to high levels of contamination in various environmental matrices and food stuffs. The increasing presence of these chemical residues in various commonly consumed food products is of great concern, due to potential adverse effects on humans and possible impact on sustainable development. In an attempt to estimate the impact of chemicals on human health in Nigeria, several studies have been carried out by researchers at the Laboratory of Ecotoxicology and Environmental Forensics, University of Benin. Pesticides and polycyclic aromatic hydrocarbons (PAH) have been analyzed in food products using gas chromatography (GC) equipped with electron capture detector (ECD), while the attendant health risk associated with the consumption of POPs in contaminated food stuffs have been determined using relevant risk assessment indices for cancer and non-cancer risk. Results have shown high concentrations of these persistent chemicals in several food stuffs in Nigeria, while human health risk predictions has shown that dietary exposures to POPs, increases the potential for cancer and non-cancer effects in humans after consumption. Therefore, there is the need for a concerted effort by relevant authorities in ensuring that chemical use is regulated while the ban on POPs is enforced.

WE224

Consumption of canned foods in Nigeria: assessing the associated human health risk.

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Ensuring food safety to protect public health and promote economic development remains a significant challenge in both developing and developed countries. In Nigeria, the changing lifestyle of people has resulted in increased consumption of canned foods which makes it imperative to determine the levels of chemical additives to avert health risks associated with consumption. To this end, levels of food additives (Nitrite and Nitrate, Sodium Chloride) and heavy metals (Fe, Mn, Zn, Cd, Pb, and As) in canned foods frequently consumed in Nigeria, were assessed to estimate human health risks associated with consumption. Chemical additives and Heavy metals were assessed using Perkin-Elmer spectrophotometer and Atomic Absorption Spectrophotometer (AAS) respectively. Human Health Risk estimates were obtained using standard indices (Estimated Daily Intake (EDI), Target Hazard Quotient (THQ), Hazard Index (HI), and Dietary Exposure (DE)). Results showed that the concentrations of Nitrate, Fe and Cd in all the canned food categories exceeded recommended limit set by the European Union, indicating potential health risk. Further risk estimations showed EDI values for Cd in all the canned food categories above the tolerable daily intake, while DE for Fe in canned sweet corn, Fe, Zn and Pb in canned beans/peas had values above recommended limits. THQ values for all the canned foods were less than 1 except canned beans/peas, while HI was above 1 in the canned fish category. This implies that there is risk of non-carcinogenic health effects from the consumption of canned beans/peas. Therefore, it's imperative that there should be constant monitoring of food additives and heavy metals in canned foods.

Pollutant risks to amphibians and reptiles: how much we know and what we need (P)

WE225

Amphibians and Plant-Protection Products - What Research and Action Is Needed?

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The majority of Swiss amphibians are at risk. There is a large number of factors that are discussed as possible causes, including plant-protection products (PPP), although to date we have not been able to adequately quantify the impact of the latter. Since the influence on amphibian populations of PPP's in the form of active ingredients, wetting agents, breakdown products or mixtures has not yet been studied to any great extent, a workshop was held with over 30 invited experts from authorities, academia, industry, agriculture, environmental NGOs, Swiss amphibian research institutes as well as environmental law. A majority of the participants deemed further research on the influence of plant protection products on amphibians appropriate. Identified research issues were: What is the exposure of amphibians to PPP in natural environments? How great is the risk to which species and developmental stages in what crops from what PPP? What effects on individuals are relevant at the population level? What population models can be used to estimate the effects observed in the laboratory with individuals, at the population level as a whole? What effect do co-formulants have on toxicity? How high is the variability in sensitivity between the various amphibian species and populations? Can entrance criteria be developed in order to identify potentially problematic substances for amphibians for the ecotoxicological risk assessment in the authorisation of PPP's? What risk-management options are there both inside and outside of the field? How relevant are other factors for the decline in the amphibian population, compared to PPP's? A further topic of discussion was how to better protect amphibians from PPP's by reducing their exposure on open ground. Discussed measures were: Avoidance of PPP application during migration in affected areas with the help of prediction models Recommendation for not using specific products Advice and awareness-raising for farmers Advice and awareness-raising for private users in their home gardens Compensation measures (e.g. amphibian-specific ecological compensation areas) Promotion of amphibians via direct payments and/or existing label schemes, e.g. IP-Suisse Reduction of PPP use through alternative plant-protection strategies

WE226

Incorporating Habitat Variables into a Weight-of-Evidence Analysis of Amphibian Risks in Wetlands

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Weight-of-evidence analyses may include a comparison of ambient chemical concentrations in the environment to environmental guidelines, field survey results, and toxicity test results. Risk assessment of amphibians in wetlands can benefit from also considering habitat variables, as these can have a profound influence on populations and communities. The current study compared metal concentrations in water collected from eight wetlands potentially influenced by industrial emissions, and four reference wetlands, to guidelines and amphibian toxicity data collated from the literature. Amphibian diversity and abundance data for these same wetlands also were collected. Statistical analysis of habitat variables relative to amphibian occupancy and relative abundance was completed for these and approximately 40 other wetlands in the area. Including the habitat evaluation contributed to understanding the potential risks to amphibian populations in wetlands near the facility. The analysis showed that, although concentrations in water exceeded guidelines for several metals/metalloids, concentrations generally were below amphibian toxicity effect levels. Habitat variables and life history characteristics of the amphibians were stronger indicators of population presence and abundance than metal concentrations.

WE227

Evaluation of effects of herbicide products and their active substance on amphibian: Tests of glyphosate exposure on the development of common toad (Bufo bufo)

J. Sadowski, F. Göge, JKI; A. Esther, JKI / Vertebrate Research

Common toad (*Bufo bufo*) is a typical species of the European agricultural landscape and potentially threatened by pesticide exposure. Even modern perennial agrochemical use is accompanied by a high exposure risk. However, studies on the influence of pesticide exposure to European amphibian species are rare. For that reason we tested in an acute toxicity study the influence of the herbicide Roundup® PowerFlex and its active ingredient (a.i.) glyphosate in high and environmentally relevant doses on survival and development of Common toad. In the laboratory, eggs were exposed to Roundup® PowerFlex or glyphosate for 24h or to compare with untreated control groups. Rate of hatching individuals and size, weight and survival rate during time to metamorphosis were determined. The preliminary results showed that high dose treatments let to expected severe effects on toad's development. Environmental relevant doses seemed to have no effects, but more replications would be necessary. Systematic toxicity studies are necessary to assess the risk of European amphibian species due to the lack of suitable and comparable data. Standards for laboratory tests should be developed and single and mixed pesticide exposure scenarios on environmentally relevant level should be done for risk assessment of different species living in the current agricultural landscape.

WE228
Cadmium but not Lead affects Xenopus laevis fertilization and embryogenesis.
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Environmental contaminations have significant effects on biodiversity, especially on Amphibians group. This class, containing the highest number of known threatened species, is highly sensitive towards chemical contaminants. Despite toxicity, dominance and persistence in the environment of metal contaminants, effects on amphibians remain poorly documented, except through the FETAX test. But this assay, use to determine lethal and teratogenic concentrations of solutions, runs with few-days old tadpoles. So effects on gametes, external fertilization and embryogenesis are not considered. Whereas these steps are also exposed to environmental contaminations, they're stills poorly documented yet. In this context and according to previous results of ours showing that *X. laevis* oocytes maturation was altered by metal chlorides, we examined how cadmium and lead interact with earlier stages of *Xenopus laevis*, from fertilization to 8-cells zygotes. While lead doesn't disturb the fertilization rates, cadmium decreases these reproductive parameter in a dose dependant manner. Similar harmful effects are obtained when these two metals are in mixture at 25 mg/L. Whereas more investigations are required, at the sight of these outcomes, we could assume there is no combination effect when cadmium and lead chlorides are in mixture. We also observed that cadmium delays the occurrence of different embryogenic stages. So it disturbed cell division. These observations are in accordance with previous studies analysing from a histological point of view the *Xenopus* oocyte maturation in cadmium contaminated media, where different kinds of atypical structures on the chromosomal formations (pronuclei, ectopic and disorganized spindles) have been reported. Cadmium also decreases fertilization whatever the male or the female gametes have been pre-exposed. But the fertilization success is more affected when oocytes are the Cd pre-exposed ones. Furthermore, no fertilization are able to reach the stage 2 in this condition. Our results highlight that cadmium altered *Xenopus* reproduction, affecting fertilization success and embryogenesis, preventing the formation of tadpoles. Analysing earlier stages will procured complementary information to the FETAX test. Moreover oocytes seem to be particularly threatened, and let suggest they could be considered as early and sensitive endpoint (and target) to assess environmental contaminants effects.

WE229
Effects of environmental stressors on the amphibian' pathogen Saprolegnia australis
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Saprolegniasis is the common term used to report oomycete water mold infections. They have been responsible for large economic losses on aquaculture due eggs and fish larvae infection. As well, amphibian natural populations are also strongly affected by such pathogenic agents. Water mold infections have been documented and linked to amphibian population declines worldwide. These fungus-like organisms are in their majority saprobes, but are capable of parasitism and may act as opportunistic pathogens (e.g.cutaneous injuries). Besides, effects of water molds on amphibians appear to be influenced by several environmental factors, including temperature, pH and ultraviolet-B radiation. The present work aimed at understanding the effects that environmental changes may cause on amphibian pathogens. To achieve this objective, the pathogenic agent, *Saprolegnia australis*, was exposed to different levels of NaCl, seawater and acid mine drainage (AMD). Ecotoxicological assays were carried by exposing the mold, in PGA culture medium with different NaCl concentrations (ranging from 4.9 $mScm^{-1}$ to 54.9 $mScm^{-1}$), AMD dilutions (with dilutions ranging from 0% to 100%) and seawater (concentrations equivalent to NaCl solutions conductivity and the highest tested concentration being the one corresponding to SW). At the end of the assay, vegetative growth, biomass production, extracellular enzymatic activity and mycelium chemical composition were assessed in *S. australis* exposed under control and contaminated conditions. Results from mycelial growth of *S. australis* showed no effect on vegetative colony growth exposed to NaCl. However, a significant growth inhibition relatively to the control was observed when the mold was exposed to 0%, 25%, and 50% of AMD effluent dilution. For biomass production, results showed that NaCl and SW appears to have similar effect concentration values (EC_{20}= 47.0 $mScm^{-1}$ and EC_{20}= 41.7 $mScm^{-1}$ respectively). Regarding the quantity of biomass produced under effluent exposure, a reduction of 80% was observed at the AMD dilution of 75%. NaCl and AMD effluent also exerted effects on extracellular enzymatic activity. Under NaCl exposure, the water molt increase the gelatin and casein degradation and decrease lipase activity on the highest concentration. In general, effluent decreased the degradation of used substrates.

WE230
Biomarkers of oxidative stress in lizards from crops with different management intensity
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Reptiles are an understudied group in ecotoxicology and little is known about the effects of pesticides on them. Several studies have shown the presence of some species within crop fields, with the consequent risk of becoming exposed to pesticides, and also that increasing intensity of agricultural management could compromise fitness in several lizard populations. The metabolism of pesticides in the organism produces reactive oxygen substances (ROS), which are normally neutralized by the antioxidant system. When the production of ROS surpasses the capacity of this system, the non-neutralized ROS may damage different structures of the cells, causing oxidative stress. We compared oxidative stress biomarkers among populations of two species of lizards (*Podarcis siculus* and *P. muralis*) inhabiting olive orchards and vineyard with a different degree of management (from organic fields to intensively managed crops) at Montepaldi Farm (Tuscany, Italy). We did not find any effect of species or gender on biomarkers, but the tail integrity influenced the outcome of glutathione concentrations and body condition. TBARS, an indicator of lipid peroxidation, increased concentration was associated with increased intensity of management in olive orchards. The enzymatic activity of glutathione peroxidase was reduced with respect to organic fields in olive orchards with an intermediate management intensity but not in those intensively managed, which could indicate a consumption of the enzyme at low pesticide concentrations that would eventually end up in new synthesis under increasing concentrations. However, responses of these two variables were only significant when considering lizards with regenerated tails, but not in animals with intact tails. A possible explanation for this result could be that of an energetic trade-off between the regenerative process and the antioxidant system, however further research is needed to unveil such kind of interaction. Our results highlight the importance of running integrated assessments of population health in order to understand the impacts of agricultural intensity in reptiles. The lack of basic ecotoxicological data and standard test methods for reptiles makes especially interesting this type of integrated field approaches for pesticide risk assessment in reptiles

Science based strategies for the environmental assessment and management of pharmaceuticals and veterinary medicines (P)

WE231
TOXICITY EVALUATION OF OZONE-TREATED WASTEWATER FROM A PHARMACEUTICAL MANUFACTURING FACILITY
J.G. Tell, Merck & Company, Inc. / Global Safety the Environment; R. Watts, K. de Bruin, Merck Sharp & Dohme; A. Vlaski, A. Haener, ARCADIS; J. Rijk, M. Migchielsen, WIL Research Europe BV
MSD is currently implementing a multi-year initiative to improve wastewater treatment and implement water sustainability projects at manufacturing sites worldwide. As part of the improvements, a pharmaceutical formulation site is installing two new wastewater treatment plants (WWTPs) for waste streams containing Active Pharmaceutical Ingredients (APIs) from two production areas before discharge to the municipal WWTP. Ozone was identified as a potential Best Available Technology (BAT) for treating a broad spectrum of APIs and was confirmed to be feasible for this application based on waste stream characteristics. Bench-top testing of ozone treatment was conducted using collected waste stream samples from the two production areas to verify removal of APIs below the required discharge concentrations established for no environmental impact. Based on the testing, an optimal ozone dose range and contact time were established (range of 50 mg O_3/L at 30 min contact/reaction time to 100 mg O_3/L at 15 min contact/reaction time). The potential negative impact of ozone treatment on water quality, specifically related to toxicity of the future treated effluent discharges, was addressed in a toxicity testing program. This included the following toxicity analyses for waste streams from the two different discharge points before and after

bench-top ozone treatment: Algae and *Daphnia* toxicity testing Estrogenic and Androgenic hormonal activity testing Bio-respirometry and 21 days DOC elimination testing. All toxicity, hormonal activity and biodegradability tests indicated the reduction of toxic effects in the tested waste streams after ozone treatment. These results are in conjunction with the observed API removal and confirm ozone as BAT for treatment of these API-containing waste streams.

WE232
EFFECTIVE REMOVAL OF ACTIVE PHARMACEUTICAL INGREDIENTS FROM INDUSTRIAL WASTEWATER
R. Watts, M. Collett, Merck Sharp & Dohme; P. Ross, M. DeMarco, ARCADIS; D. Vughs, KWR Watercycle Research Institute
MSD is currently implementing a multi-year initiative to improve wastewater treatment and implement water sustainability projects at manufacturing sites worldwide. As part of the improvements an initial literature screening on Best Available Technology (BAT) for the removal of Active Pharmaceutical Ingredients (APIs) was completed. Numerous articles describe possible techniques for the removal of APIs from wastewater, and ozone is generally reported as an efficient API removal/destruction technology. However, a range of ozone dosages up to 120 mg/L are reported depending on the wastewater matrix. In general, the rule of thumb for ozone (O_3) dosage would be a factor 3 or 4 to 1 per unit of chemical oxygen demand (COD). For industrial wastewaters with a high COD, this would result in very high ozone dosages which can translate into high investment and operating costs. During this study several industrial wastewater streams, with varied compositions including 20 different APIs, were sampled and bench scale tests with ozone were performed. A dosage range of 0.01 to 4.4 mg O_3/mg COD was applied during testing. For each API, a method was developed using Ultra Performance Liquid Chromatography – Mass Spectrometry (UPLC-MS/MS) to analyze the APIs in wastewater at concentrations as low as 0.1 μg/L. Results showed that good API removal efficiencies could be achieved for wastewater matrix COD levels as high as 5300 mg/L. Counter to the general perception, it was found that low dosages of 10 mg-O_3/L yielded as much as 70% removal for some APIs. Extreme ozone dosages of up to 3000 mg O_3/L resulted in 99.999% removal of specific APIs in highly concentrated waste streams. Ozone treatment provided the required removal efficiencies for 18 of the 20 APIs tested. Widespread application of effective API removal from wastewater remains a major challenge. However results from this work show that ozone treatment can be an effective solution for a range of APIs and wastewater matrices. The required dosages for API destruction were lower than initially expected, resulting in a cost effective solution.

WE233
Experience with AstraZeneca's Environmental Reference Concentration (ERC) Policy - Managing API Emissions for Internal and External Manufacturing Facilities.
B. Dillon, AstraZeneca / AstraZeneca Global Environment; K. Hutchinson; C. Hargreaves, Astrazeneca UK Ltd / AstraZeneca Global Environment
The issue of Pharmaceuticals in the Environment has received increasing attention in both academic literature and wider media of recent. Whilst Environmental Risk Assessments are required for patient use of new medicines as part of the regulatory submission to health authorities within the EU and USA, there remains a lack of guidance associated with potential impact from Active Pharmaceutical Ingredient (API) emissions from manufacturing. In this poster, we outline AstraZeneca's Environmental Reference Concentration (ERC) concept and its application to manufacturing sites both internally and externally as a tool for the risk management of API discharges to the aquatic environment. The Environmental Reference Concentration is defined as "the average concentration of an API in the receiving surface water environment that, based on current scientific knowledge, would be unlikely to result in any adverse long term effects". AstraZeneca have previously published our approach to monitoring emissions through the ERC procedure.[1] This poster aims to highlight our application and experience of the procedure *via* both practical and theoretical risk based assessments from both internal sites and external Contract Manufacturing Organisation (CMO) suppliers. [1] Murray-Smith R, J; Coombe V, T; Grönlund M, H; Waern F; Baird J, A. *Integr Environ. Assess. Manag.* **2012** Apr;*8*(2):320-30.

WE234
Toxicity assessments of the selected sulphonamides solutions after ozonation in comparison to oxidation by fungal peroxygenase
N. Lemańska-Malinowska, Environmental Biotechnology Department; Z. Gajda-Meissner, T.F. Fernandes, Heriot-Watt University / School of Life Sciences
Veterinary drugs from the sulphonamides group (SAs), such as sulfamethazine (SMZ) and sulfadiazine (SDZ), are the oldest chemotherapeutic agents used for antimicrobial therapy and due to their bioactivity, they still play an important role. Very good results in the decomposition of these drugs in the aquatic environment were achieved by ozonation and using an oxidation process with unspecific peroxygenase from *Agrocybe aegerita* (*Aae*UPO). Nonetheless, special attention should be paid to the risk of by-products' formation, which may cause potential toxic effects on the living organisms. Therefore, the aim of this study was to compare the acute toxicity of SMZ and SDZ before and after both processes in a range of trophic levels. For this purpose, toxicity tests on *Vibrio fischeri*, *Pseudokirchneriella subcapitatum (CCAP 278/4)* and *Daphnia magna* were conducted, as described below. Toxicity tests on *Vibrio fischeri* bacteria were carried out using Microtox® analyser according to the Whole Effluent Toxicity (WET) test procedure. The effect of test solutions on the growth of the *Pseudokirchneriella subcapitatum (CCAP 278/4, green algae)* was measured as the rate of change of biomass. *Daphnia* acute toxicity tests were performed according to the OECD guideline 202 as range finding and definitive tests. The 24 h and 48 h EC_{50} values were calculated using SigmaPlot® software. The results obtained from toxicity tests were compared. No EC_{50} values could be calculated for the test with bacteria. SDZ and SMZ caused 50% of algae growth inhibition after 72 h of exposure at concentration 4.95% v/v and 6.54 % v/v respectively. EC_{50} values after 72 hours were 9.82% v/v and 9.83% v/v. For the freshwater crustacean the EC_{50} values were in the range 25.98- 33.55 %. It is concluded, that the ozonation process significantly decreased toxicity of the samples. For the enzymatic oxidation of selected SAs tests on *V. fischeri* showed a EC_{50} equal to 50.69 % for SDZ solution after this process was applied. SDZ and SMZ caused 50% of *P. subcapitatum* growth inhibition after 24 hours of exposure at concentration 5.14% v/v and 4.83 % v/v, respectively. After 72 h exposure the growth of algae was almost completely inhibited. EC_{50} values for *D. magna* test were in the range 14.54- 15.37 %. In general, SMZ and SDZ mixtures before and after ozonation are much less toxic when compared to the enzymatic oxidation process.

WE235
Difference of physiological activities of pharmaceuticals of wastewater between UK and Japan
H. ZHANG; M. Ihara, S. Hanamoto, N. Nakada, Kyoto University; M.D. Juergens, Centre for Ecology and Hydrology / Wallingford; A. Johnson, CEH Wallingford / Wallingford; H. Tanaka, Kyoto University
Pharmaceuticals have been widely detected in water environment at low concentrations. However, concern has been raised on their potential risks to human and aquatic species, because they are designed to be biologically active. To better understand this concern, we must know the physiological activity of pharmaceuticals in waters based on their modes of action (MoAs). One of the most important MoAs is interaction with G-protein coupled receptors (GPCRs). Untill now, we have detected physiological activities of GPCR-acting pharmaceuticals against some class of GPCRs in secondary effluent (SE) of wastewater treatment plants (WWTPs) in Japan by using *in vitro* transforming growth factor-α (TGFα) shedding assay. However, the situation in other countries is still unclear. In this study, we measured and compared the physiological activities of GPCR-acting pharmaceuticals in SE of WWTPs in UK and Japan by using *in vitro* TGFα shedding assay. We measured the antagonistic activities of pharmaceuticals against angiotensin (AT1), dopamine (D2), acetylcholine (M1), adrenergic (β1), and histamine (H1) receptors. Activities detected in waters were quantified as antagonist equivalent quantities (EQs). Concentrations of sulpiride (antagonist for D2 receptor) and metoprolol (antagonist for β1 receptor) were analyzed by LC-MS/MS in parallel. We compared the EQs, as measured by the TGFα shedding assay, with concentrations of corresponding pharmaceuticals, and investigated whether measured antagonistic activities in the wastewater could be explained by these known pharmaceuticals. In addition, in order to investigate more detail how different physiologocal activities of pharmaceuticals in SEs between UK and Japan, we fractionated SEs, and compared the EQs in each fraction between UK and Japan sample. If the fraction in which highest EQ is detected is different between UK and Japan samples, it means that corresponding pharmaceuticals for detected EQs are different between UK and Japan.

WE236
Case Studies Demonstrating the Advancement of Wastewater Management in the Pharmaceutical Industry
J.G. Tell, Merck & Company, Inc. / Global Safety the Environment; D.J. Caldwell, Johnson & Johnson
Across the human health pharmaceutical industry, we make a vast and diverse portfolio of products, each of which can have environmental risks. Good stewardship includes understanding the potential environmental and social implications and employing life-cycle thinking and tools to minimize any potential environmental risks – from the early stages of product design, to formulation and manufacturing, to the product's impacts during use and final disposal of residual product. EcoPharmacoStewardship is the framework that applies the widely-accepted principles of product stewardship. The three pillars of the framework are extended environmental risk assessment (eERA), extension of the scientific knowledge base (IMI-iPiE), and manufacturing effluent management, which is the focus of this poster. In 2011, a group of major pharmaceutical companies met to initiate a formal dialogue on the issue of best practices for managing pharmaceutical discharges from site operations. The companies shared their current programs at the time and embraced the concept of a maturity ladder that provides a mechanism for benchmarking across the industry (Caldwell, et al. 2015). Recently, these same companies reconvened to evaluate where each currently stands along the maturity ladder and to discuss changes and improvements to company-wide programs over time. This poster summarizes

some of the advances made by the pharmaceutical industry and the area for future work.

WE237
Perspective and challenges for generation of effects and fate data on innovative veterinary API
C. Durou, CEHTRA SARL; R. Magnier, A. Bauer, Ceva Santé Animale; B. Journel, CEHTRA SARL
An environmental impact assessment (EIA) has to be performed on the active pharmaceutical ingredient (API) for the market authorisation of veterinary medicines. The scientific guidelines are made publicly available by the EMA; among others, the guideline lists the minimum data requirements for the assessment of PBT/vPvB properties and how to conduct an EIA. The generation of fate and effect data is an iterative process. In a first Tier (A), the data requirements include the determination of physical-chemical properties (e.g. water solubility, n-octanol/water partition coefficient, fate properties (adsorption/desorption, route and rate of degradation) and short-term ecotoxicity dataset. In a second Tier (B), higher tier testing is required for fate (e.g. bioconcentration) and effects (long-term ecotoxicity) are requested. Finally, specific data may be requested on a case-by-case. For any new API, the available dataset on the active may be limited to the intended use of the VPI, pharmacological information and mode of action. While such data are important for the purposes of identifying emission route to environment and for designing an appropriate testing strategy, further information are necessary. Firstly, physico-chemical constitutes a key initial step given their importance as input data for exposure assessment and also as prerequisite for fate and effect data. As an example, behaviour and stability of new API in contact with water or standard aquatic test medium is missing at initiation of test program. Also, due to the mode of action but also in case of continuous emission to environment, long term toxicity studies may be preferred to short term studies. Next to these data gaps, the new API may exhibit one or more difficult to test properties for example, ionisation status is a common difficult to test property. Considering the overall, there is a need to order testing, allows flexibility in test program. The more relevant and reliable data are, the more robust is the environmental impact assessment. The purpose of this work is to initially review the data requirement for an EIA for a veterinary API which miss basic data need to define the suitable test method and test strategy.

WE238
Parasiticides - highly used veterinary pharmaceuticals and their impact on the environment
N. Adler, UBA FG IV 2.2 Arzneimittel; I. Ebert, Umweltbundesamt / Pharmaceuticals Department; J. Schoenfeld, Federal Environment Agency
Several groups of antiparasitic active substances are marketed and often used in food animals. While parasiticides are highly potent to eliminate endo- and ectoparasites, they have also effects on non-target organisms, such as dung flies and beetles and water flees. Environmental risk assessments that are submitted during the authorization process of veterinary pharmaceutical products show high risks for non-target organisms. In addition to these risks a parasiticide poses to the environment, some of these active substances are assessed to be hazardous. However, no products containing antiparasitic substances were refused to enter the market, so far. Instead, risk mitigation measures are defined to reduce the risks to an acceptable level. The poster presents risk assessments and the hazard characterization of different parasiticides and it will further discuss the defined risk mitigation measures regarding their suitability as well as the decisions made by regulators.

WE239
Methodology for considering the environmental risks of parasiticides in a risk-benefit assessment
J. Chapman, Environmental Department University of York; A. Boxall, University of York / Environment Department; C. Sinclair, The Food and Environment Research Agency / Centre for Chemical Safety and Stewardship; P. Howley, University of York; G. Jones, The Food and Environment Research Agency
As part of the authorization process, veterinary medicinal products (VMPs) require a benefit-risk assessment, which includes consideration of environmental risks. However, the environmental risks are not directly comparable to the therapeutic benefits (i.e., efficacy). In this study, we therefore developed an approach for communicating environmental risks of VMPs in a benefit-risk assessment. We investigated the risks 3 parasiticide compounds (ivermectin, moxidectin and fenbendazole) using summaries of product characteristics (SPCs), literature sources, and regulatory guidelines. To facilitate the benefit-risk comparison, a visual decision support matrix was developed and applied to each compound. We found the environmental risk matrix highlighted key tradeoffs and discrepancies in available data and could therefore be a valuable decision support tool. The improvement of methods to consider environmental risks in the benefit-risk assessment will aid decision-makers to select to best option and make the authorization process more transparent.

WE240
Towards an updated ERA for propranolol
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Propranolol is a non-selective adrenoreceptor blocker (β-blocker) and is prescribed to treat high blood pressure, cardiac arrhythmias, glaucoma, anxiety and migraines. As a result of its widespread use and pseudo-persistence propranolol is frequently detected in European sewage effluents and surface waters. Although an environmental risk assessment (ERA) undertaken according to European Medicine Agency (EMA) guidelines exists, and PNEC values for propranolol have previously been published (Murray-Smith et al. 2011), it is recommended that these are reviewed at regular intervals (Holm et al., 2012; Ågerstrand et al. 2015). A considerable quantity of new environmental effects and monitoring data for propranolol has been published in the scientific literature since the original assessment was conducted. We therefore present an updated ERA for propranolol, also conducted according to EMA guidelines, but taking account of all environmental fate and effects data published in the scientific literature to date. This will include an assesment of the measured environmental concentration (MEC) data for propranolol in both treated sewage treatment plant (STP) effluents and surface freshwaters. These will be compared with the predicted environmental concentrations (PECs) derived based on usage and dose data. Updated freshwater PNEC values for propranolol will also be presented, applying both deterministic and probabilistic derivation approaches. Finally, the MEC and PNEC data will be used to present updated Risk Characterisation Ratios (RCRs) for a series of different freshwater exposure scenarios for propranolol. Murray-Smith RJ, Coombe VT, Grönlund MH, Waern F, Baird JA. 2011. Managing emissions of active pharmaceutical ingredient from manufacturing facilities: An environmental quality standard approach. Integrated Environmental Assessment and Management 8:320–330 Holm G., Snape JR., Murray-Smith R., Talbot J., Taylor D., Sorme P. 2013. Implementing Ecopharmacovigilance in Practice: Challenges and Potential Opportunities. Drug Safety, 36(7):533-46. Ågerstrand M, Berg C, Björlenius B, Breitholtz M, Brunström B, Fick J, Gunnarsson L, Larsson J, Sumpter JP, Tysklind M, Rudén C. 2015. Improving environmental risk assessment of human pharmaceuticals. Environmental Science and Technology. 49 (9):5336–5345

WE241
Review of ERA data submitted as part of pharmaceutical marketing authorisations
D. Leverett, WCA-Environment Ltd; R. Marks, M. Gross, WCA Environment; J. Snape, AstraZeneca UK Ltd. / AstraZeneca Global Environment; K. Hutchinson,
According to European Medicine Agency (EMA) guidelines, most marketing authorisation submissions for new pharmaceutical products require an Environmental Risk Assessment (ERA) of the active pharmaceutical ingredient (API) in the product, which should be submitted as part of the application. Exceptions to this requirement include so-called biologics (e.g. proteins, peptides, vitamins) where sufficient justification can be provided on a case-by-case basis that there is likely to be no significant environmental risk, and synthetic APIs where it can be shown that the new product will not lead to an increase in environmental exposure to the API. The ERA is conducted in two phases, with those substances meeting the criteria for further evaluation following a Phase I screening assessment proceeding to Phase II. In Phase II, a set of environmental fate and aquatic toxicity studies are required and a refined risk assessment for any compartments of concern may then need to be conducted in a Phase II, Tier B assessment. For products for which an EU-wide marketing authorisation is sought, the outcomes of each ERA are presented in a European Public Assessment Report (ePAR) which is publically available on the EMA website. However, the level and type of ERA information presented for each product can be extremely variable (especially for those applications submitted before 2006, when the current EMA guidelines were published). There may be no ePAR available at all, and ERA information contained in ePARs can vary from simple statements to a full presentation of hazard and exposure data. In addition, for generic APIs (i.e. those outside of patent) there are often a large number of applications (and therefore ePARs) for the same API. Nevertheless, a potentially large amount of environmental hazard data for APIs is available via these documents, and by cross-referencing the information on the EMA website for different products it may be possible to compile API hazard data, and potentially derive tentative PNEC values. In this poster we will present an analysis of the environment hazard information available on the EMA website for APIs in products for which an EU-wide marketing authorisation has been granted. In particular we will show data on the products for which a Phase II ERA was conducted, including (where possible) the environmental hazard data. We then present an evaluation of the hazard data for each API, and attempt to derive tentative PNECs using this data, according to EMA guidance.

WE242
Science-based Approaches to prioritise the Environmental Risks Posed by Legacy Human Medicinal Products

J. Snape, AstraZeneca UK Ltd. / AstraZeneca Global Environment; S. Owen, AstraZeneca / Safety Health Environment; L. Gunnarsson, University of Exeter / Biosciences; A. Boxall, University of York / Environment Department

There are approximately 1500 Active Pharmaceutical Ingredients (APIs) in use today. However, the environmental risk of only a small proportion of these APIs has been assessed. Our ability to detect trace levels of pharmaceuticals in environmental matrices is also outpacing our capacity to assess these data within the context of environmental risk. Consequently, there is a real need for scientifically sound and pragmatic approaches to prioritize legacy APIs based on potential environmental risk for additional experimental testing or assessment under established regulatory guidelines. Over the past decade, several prioritization approaches have been proposed, primarily by the research community, for use on APIs and these have been used to identify substances for further scrutiny. These approaches vary in the methodologies used: some are exposure-based, some hazard-based and some take a risk-based approach; the majority of approaches have focused on aquatic exposures with only a few considering potential impacts in terrestrial and benthic environments; few have considered exposure of birds and mammals; and general approaches have been restricted to specific geographical regions and environmental compartments. As existing experimental data are only published for a small proportion of pharmaceuticals in use, most prioritization processes rely on existing data or information on read across from the human pharmacology and pre-clinical safety data and/or predictive models such as quantitative structure-activity relationships (QSARs). While our knowledge around human to environment read-across has increased over the past few years, there are still many uncertainties. The applicability of many of the predictive models that have been applied is questionable, particularly given that the majority of APIs are ionogenic in nature while most predictive models in use have been developed for neutral organic compounds. This presentation outlines a potential approach, that meets the criteria described above, that could be employed by the iPiE project. This approach pulls together some of the approaches that have been used previously but is much more holistic in terms of the compartments and risk-endpoints considered. It is our expectation that the approach could be parameterised and applied to different geographical regions to reflect differences in the API emission pathways to different compartments into the natural environment, levels of treatment, treatment connectivity and characteristics of the receiving environment.

WE243

QSAR Studies on the Potential Environmental Hazard of Pharmaceuticals

A. Sangion, P. Gramatica, University of Insubria / DiSTA

Active Pharmaceutical Ingredients (API) have become ubiquitary present in the environment, for this reason in 2006 the European Medicines Evaluation Agency (EMEA) published guidelines for the environmental risk assessment of human pharmaceuticals. Every environmental risk assessment (ERA) requires large amounts of data for each chemical but, unfortunately, information on PBT behaviour and ecotoxicological data are available only for a little percentage of API in literature and databases. We propose the characterization of the intrinsic hazard potential of pharmaceuticals by QSARs development and application. Some pharmaceuticals have been prioritized as potential PBTs by both Insubria PBT Index in QSARINS and US-EPA PBT Profiler in consensus approach. Then, from literature, we collected and carefully curated datasets for ecotoxicity data of species at different trophic levels (algae, Daphnia, fish) in order to consider a simplified aquatic ecosystem. For each species we developed ad hoc QSAR acute toxicity models, based on PaDEL molecular descriptors using OLS method and Genetic Algorithm for Variable Subset Selection in QSARINS software. All models are robust ($R^2 > 0.75$, $Q^2_{loo} > 0.70$) and externally validated (CCC> 0.85) on different splitting schemes. The structural Applicability Domain (AD) of the proposed models to pharmaceuticals without experimental data has been verified and demonstrated to be very high with the 74% of chemicals inside the AD for all the toxicity models. Moreover, reliable predictions from different models applied on a set of more than 1000 pharmaceuticals were combined by Principal Component Analysis (PCA) to find an ecotoxicity trend representative of the pharmaceuticals' toxicity in the whole aquatic ecosystem. This trend, called overall aquatic toxicity index (ATI), has been then modelled by molecular descriptors to obtain a QSAR model useful to highlight, directly from the chemical structure, the pharmaceuticals potentially most hazardous for the environment. This index and the predictions obtained by it could be used to refine procedures of input prevention and control at consumer level as well as *a priori* in the rational design of environmentally safer pharmaceuticals. Finally, we propose also interspecies correlation models to extrapolate information from one simpler trophic level (such as Daphnia) to another more complex (Fish), reducing the use of animal tests.

WE244

Effects of the beta-blocker metoprolol and the NSAID diclofenac on the embryonic development and health of brown trout Salmo trutta f. fario

H. Schmieg, Tübingen University / Animal Physiological Ecology; S. Schwarz, University of Tuebingen / Animal Physological Ecology; M. Scheurer, Water Technology Center TZW Karlsruhe; H.-. Köhler, University of Tuebingen / Institute of Evolution and Ecology Animal Physiological Ecology; R. Triebskorn, University of Tuebingen / Animal Physiological Ecology

The discharge of human and veterinary pharmaceuticals into surface waters via wastewater treatment plants is a growing problem in Central Europe. However, despite the frequent detection of various substances in aquatic ecosystems, knowledge on their effects in biota is still scarce. Available data are based on standard biotests with model test organisms, which often lack ecological relevance. Two of the most frequently used pharmaceuticals and, hence, also frequently detected in surface waters, are the beta-blocker metoprolol and the NSAID (nonsteroidal anti-inflammatory drug) diclofenac. Our present study investigated the effects of metoprolol and diclofenac in brown trout, *Salmo trutta* f. *fario*, a species of high ecological relevance for Central Europe. The applied concentrations were 0.1, 1, 10, 100, and 1000 µg/L for metoprolol and 0.1, 0.5, 1, 10, 100 and 200 µg/L diclofenac, thus including several concentrations in ranges reported for German surface waters. Juvenile fish (six month post hatch) were exposed for 28 days. Investigated endpoints included biometric measurements, mortality, behavioural abnormalities, stress protein level (Hsp70) as a biomarker for proteotoxic stress and degree of lipid peroxides as a biomarker of oxidative stress. Furthermore, histology of liver, kidney, gill, and heart was assessed reflecting the overall health condition of trout. Additional tests with trout embryos and sac-fry stages were based on OECD guideline 212. Recorded endpoints in these tests were mortality, developmental parameters and histological condition. The diclofenac exposure of juveniles led to significantly increased mortality and to behavioural abnormalities already at low concentrations (0.1 µg/L). No such effect was seen for metoprolol. Furthermore, the stress protein level and the histology of liver and heart of trout remained unaltered. However, the erythrocyte to lymphocyte ratio in the heart of metoprolol-exposed brown trout was reduced compared to the control. Histological and biochemical investigation of the juvenile trout and fry will complement the study. Overall, our study aims at providing further information on the effects of environmentally relevant concentrations of frequently used pharmaceuticals in a species of high ecological relevance in Germany and adjacent countries.

WE245

Combined Environmental Risk Assessment for the Antiviral Pharmaceuticals Ganciclovir and Valganciclovir

J. Straub, F.Hoffmann-La Roche Ltd / Roche Group Safety Health Environmental Protection

An environmental risk assessment (ERA) was conducted for the antiviral, primarily anti-cytomegalovirus active pharmaceutical substances ganciclovir (GCV) and valganciclovir (VGCV). VGCV is the L-valine ester of GCV and a prodrug for the latter, formulated for better oral bioavailability; hence, the ERA focusses on GCV. Predicted environmental concentrations (PECs) for GCV were calculated for the relevant environmental compartments sewage treatment, surface waters and sediment, based on documented use data for both GCV and VGCV for Europe. Predicted no-effect concentrations (PNECs) for the same compartments were derived from chronic ecotoxicity tests. The ERA compares PEC with PNEC per compartment in the form of a risk characterisation ratio. Moreover, based on mutagenic and reprotoxic effects in mammals GCV is a CMR (carcinogenic, mutagenic, reprotoxic) substance; hence the fish early life stage test was extended to encompass reprotoxic endpoints. A PBT (persistence, bioaccumulation and high ecotoxicity) assessment complements the PEC/PNEC-based ERA.

WE246

Paradigm shift towards a substance-based environmental risk assessment of pharmaceuticals

I. Rönnefahrt, Federal Environment Agency / Section IV Environmental Risk Assessment of Pharmaceuticals; N. Adler, UBA FG IV 2.2 Arzneimittel

Even more than 10 years after implementing the environmental risk assessment (ERA) into the EU pharmaceutical legislation there is still a lack of data on fate and effects of active pharmaceutical substances. The reason for that is quite simple: A review program of 'legacy' products, which were approved before the ERA requirement was set, was never envisaged. But even for newly approved pharmaceuticals sometimes no full data sets essential for an ERA are available due to various reasons. In the pre-market phase an ERA is required for all new applications. This often leads to duplication of data and of assessments and hence, to a waste of resources. This may also lead to contradictory assessments and even to different risk mitigation measures for medicinal products intended for the same indication. The described problems with the functioning of the pharmaceutical legislation could largely be solved with a paradigm shift toward a substance-based ERA. One of the options which is currently discussed on an EU level is the establishment of a monograph system on active pharmaceutical substances. The aim of such a monograph system is to collate existing fate and effects data on active substances, to generate missing studies and data of high quality, to evaluate studies and to agree on the endpoints to be used for ERAs of medicinal products. Once established all authorizations of medicinal products would use the agreed endpoints from the monograph of the respective active substance to perform the ERA of the product. Overall, only a pre-market monograph systemon fate and effects data of active pharmaceutical substances in conjunction with an effective monitoring within the post-market surveillance and regular adjustments of these information to the scientific and technical progress will be able to ensure the environmental safety of medicinal products in use. This will ensure up-to-date risk assessments which are the vital base for any kind of risk management. Therefore a paradigm shift towards a substance based ERA and the collection of environmental information in substance files (monographs) will be the key measure to ensure the environmental safety of medicinal products in use. Challenges for the establishment of a monograph system are first of all clear rules on set up and management of consortia, rules on data protection, cost sharing etc. Examples of successful collaborations already exist in other legislative areas like REACH and biocides.

WE247

Mining Environmental Agency Databases: A Powerful Tool for Understanding the Fate of Pharmaceuticals and Personal Care Products

B. Hawdon, University of Portsmouth / Institute Marine Sciences; G.J. Watson, University of Portsmouth / Institute of Marine Sciences

As pharmaceutical use continues to increase so does the release of these potentially damaging compounds into the environment through treated and untreated sewage. This problem has been recognized since the early 90's but only now are policies being developed to deal with the problem. Many scientists and policy makers require more information on the long term release and fate of pharmaceuticals and personal care products. The Environmental Agency has been testing environmental samples with GCMS for a variety of pollutants since 1988 and every year more pollutants are added. Because of the Freedom of Information Act 2000 the Environmental Agency now will supply the data to the public upon request. To help direct our research we decided to test the usefulness of these databases. We requested data for variety of compounds including anti-inflammatories, anti-depressants, anti-convulsants, antibiotics, anti-cholesterol and hormone drugs for 2010- September 2015. We focussed on the Southern, Thames and Anglia region because of the diversity of populations and industries in those regions. The data we received was in Access format and contained a huge amount of information that needed some deciphering to understand what it offered. The data indicated constant collection of samples from hundreds of sites and the concentrations ranged from .001 μg to over 2000 μgs. Some of the pharmaceuticals with higher concentrations could be followed from sewage release through rivers to estuaries and coastal sites. Thus giving a better understanding of the movement and fate of these compounds. Some of the trends revealed from this data included monthly variation, annual variation, increase/decrease or constant concentrations, and geographical areas with higher levels of pharmaceuticals. At most sites the concentrations remained fairly constant, indicating flushing of pollutants, adherence to sediments or break down, but with continual releases. The mining of the databases built by the Environmental Agency through their monitoring programs is an important tool for following the fate of pharmaceuticals prescribed in the UK. This tool can be used in scientific and management collaborations to develop better policies and methods for dealing with this growing problem.

WE248

Integrated food security: mapping and selecting different indicators and metrics from sustainability till safety, veterinarian pharmaceutical case study

E. Boriani, Instituto di Ricerche Farmacologische Mario Negri; T. Hald, DTU FOOD GDSI

In food security, integration of data and knowledge across disciplines is needed to prevent food-related diseases, improve sustainability, traceability, quality, animal welfare, diminish food waste, have a clear picture of the environmental impact, improve communication to different stakeholders and introduce nutritional factors considering the enlarging need to "feed the planet". In this study, we propose a map of indicators and metrics along the production and consumption chain from "farm to fork ", in specific for an Italian pork product (Prosciutto cotto-ham), useful for decision making processes. Databases and predictive models are combined in a broad manner to find interconnections, important variables, and potential nodes to assess the overall sustainable nutrition security and improve elements such as traceability, detection of foodborne hazards, emissions in the environment, nutritious value and communication in every step of the value chain. Sustainability indicators and metrics from Life Cycle Assessment and Risk Assessment are integrated to provide a more holistic assessment of the food chain with particular attention to veterinarian pharmaceuticals case study. Certified products can gain increased credibility from the consumer, if adequate information is provided through all steps in the production chain from primary production to retail. The map considers human health risks (e.g. chemicals residues in the food, infectious agents, contaminants), benefits (e.g. nutritional values), environmental impacts (e.g. pharmaceuticals in the environment, energy consumption), and social impacts (e.g. in vulnerable population). The map helps to compare products or product chains, to identify critical steps, and to observe the problems, risks or benefit from several different perspectives. De Roest K., Pignedoli S., Belletti G., Menozzi D., Arfini F. (2014). Glamur project Italian case study: local and global cured ham chains. CRPA. Lake I.R., Hooper L., Abdelhamid A., Bentham G., Boxall A.B.A., Draper A., Fairweather-Tait S., Hulme M., Hunter P.R., Nichols G., Waldron K.W. (2012), Climate Change and Food Security: Health Impacts in Developed Countries . Environmental Health Perspectives. Vol.120, num.11.

Advancements in life cycle impact assessment method development (P)

WE249

Bridging land transformation and occupation impacts with water use in life cycle assessment: linking green water consumption with ecosystem quality impacts in Amazonia

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The framework for assessing potential impacts of land transformation and occupation (LTO) has provided valuable guidance for quantifying mid-point impacts such as changes in erosion resistance or groundwater recharge. At the same time, the inclusion of water use in life cycle assessment (LCA) has filled important modeling gaps by considering potential effects of changes in water quality and quantity on human health and ecosystem quality. We propose to bridge land use with green water consumption (as soil moisture regenerated by precipitation) in a cause-effect chain reflecting changes to the atmospheric water balance through LTO. LTO affects the amount of green water consumed by the land through evapotranspiration, which can reduce precipitation downwind (mid-point impact), thereby affecting terrestrial ecosystems which are reliant on soil moisture (end-point impact). The mid-point impact modeling step derives the amount of reduced precipitation present in a river basin based on an internal evaporation recycling ratio. The end-point impact relies on differences in climate potential species richness resulting from reduced precipitation as a step to infer ecosystem damage. We apply the proposed method to Southern Amazonia which has experienced extensive deforestation for pasture and crops and whose agricultural frontier plays an important role in changing the atmospheric water balance of Amazonia. This new framework brings closer together the effects of LTO and water consumption which are often considered separately in LCA, but whose connection is particularly important in arid and semi-arid regions as well as highly seasonal areas where evapotranspiration and precipitation are closely coupled.

WE250

Combined environmental and economical inventory for a LCA and LCCA of an insurance

I. Bosch-Frigola, F. Coca, Universidad San Jorge; J. Val, D. Chinarro, San Jorge University; M. Pino, San Jorge University / Facultad ciencias de la salud

Increasingly, the environmental characteristics of a product are analyzed not only from environmentally point of view but also economical. Therefore, combined LCA (Life Cycle Assessment) and LCCA (Life Cycle Costs Assessment) methodologies have been progressively implemented and regularly applied to industrial activities or products but less put into practice on services activities or products. This study aims to develop a combined methodology between LCI (Life Cycle Inventory) and economical inventory costs applied to the insurance policy in order to perform a LCA-LCCA coupling study. One health insurance policy from an insurance company specialized in health and prevention (DKV Seguros a member of Munich Health) was considered as functional unit. The study includes the activity of the company headquarters located in Zaragoza and 61 offices throughout Spain during 2014. The system boundaries of the study are "cradle to grave" thus incorporating not only the phase of creating insurance (business coaching, marketing and vending), but also the phase of use by insured client. Disposal stage is also contemplated. Furthermore, the inventory includes in detail all transport associated with the activity: the receipt of raw materials, transport of waste to the treatment center, transport marketing activities and the displacement of the insured client to the appropriate medical center. These environmental data have been assembled to economic data, primarily costs associated with identified flows input/outpout. The costs allocation has been carried out through an ABC system (Activity Based Costing). Data obtained from the combined inventory has allowed to connect the economic information to the environmental information at every stage and provide a basis for diagnosing the environmental impact of an insurance throughout its life cycle, as well as the costs associated with these impacts. This data analysis provided useful results to establish new strategies of prevention in the insurance sector. *This work has been possible thanks to the financial support and the systematic data provided by the company DKV*

WE251

Freshwater Ecotoxicity as an environmental impact category to guide the selection of chemical-based products

M. Claessens, Chemours; C. Cooper, International Zinc Association / Department of Marine Sciences; M. Galay Burgos, ECETOC / Environmental Sciences; R. Hoke, E.I. DuPont de Nemours and Company / Haskell Global Centers for Health and Environmental Sciences; B. Hundt, D. Kolsch, Bayer Technology Services GmbH / on behalf of Covestro; J. Lharidon, LOREAL / Life Sciences Direction; N. Otte, Henkel AG Co KGaA; F. Palais, SOLVAY; F. Schmidt, BASF Schweiz AG / Institut for Environmental Research; D. Schowanek, Procter & Gamble / Brussels Innovation Center; T. Wolf, LOreal Research / Worldwide Regulatory

Affairs for Chemistry
There is increasing interest from regulators to apply life cycle based impact assessment methodologies to assess the environmental performance of chemicals and products and to include freshwater ecotoxicity as an impact category. Examples include initiatives on green chemistry in the US as well as the Grenelle Regulation in France and the EU's Single Market for Green Products Initiative that covers both fast moving consumer goods as well as durable goods (e.g. the Product Environmental Footprint, PEF, pilot projects). A key focus area of such initiatives is the assessment of the environmental impact of chemicals and specifically the application of the USEtox method for measuring freshwater ecotoxicological impacts. The extension of the traditional LCA methodology to include the ecotoxicological impacts of chemicals raises some fundamental issues both methodologically and conceptually with the accepted norm of using risk assessment approach to manage chemicals and ecolabel consumer goods, within industry and by regulators. At the same time, the scientific committees of the EU have outlined that the current prospective environmental risk assessment methodology is far from being realistic, i.e. not delivering an accurate approximation of the environmental impact of chemicals. However, there has been no detailed assessment of whether decisions based on a CDV (Critical Dilution Volume, EU Ecolabel key criterion) or USETox comparative ranking lead to real and meaningful differences in environmental impact. With this in mind, an ECETOC Task Force was assembled to conduct a scientific evaluation of USEtox and similar tools (e.g. CDV) with the goal of establishing their relevance to the real world and to provide guidance on how to interpret their results regarding selection/ranking of chemical based products. In addition, for the USEtox methodology in particular, the influence of LCA best practices on the results of the ecotoxicological impact assessment is studied, giving attention to data availability and quality as well as treatment of missing data. Finally, a comparison of the purpose and scope of LCA and risk assessment (RA) is performed in which the potential use of specific methods from RA within LCA is explored. Guidance will be offered on how both kinds of assessment can be used in conjunction or can complement each other. This poster presents the preliminary results of this task force's activities.

WE252
Lessons from the endogenous integration of life-cycle indicators into national energy models
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Governments and decision-makers are increasingly aware of the need for assessing sustainability, which is particularly relevant to the energy sector. In this respect, life-cycle approaches are often use for the evaluation of energy systems. A key challenge is the implementation of the time dimension when using these approaches for the analysis of dynamic systems. The energy sector (both as a whole and at a lower scale) is a dynamic system. In fact, energy policies aim to guide the evolution of this system by defining performance targets e.g. in terms of greenhouse gas emissions. Energy systems modelling supports this type of policy-making process thanks to its suitability to deal with future scenarios and therefore with the time dimension. However, current modelling practices leave out a strong implementation of sustainability indicators in energy models. Within this context, the hybridisation of life-cycle and energy modelling approaches attempts to foster the strengths of both approaches while significantly overcoming their limitations. This work summarises the lessons learnt from the integration of life-cycle indicators into national energy models within the SuReTool project of the EEA/NILS Science and Sustainability programme (006/ABEL-CM-2014). The evolution of power generation in the business-as-usual scenario for Norway and Spain was analysed with a time frame from 2010 to 2050. Furthermore, the damage assessment of the involved technologies was carried out using the IMPACT 2002+ method. A number of life-cycle indicators (e.g., climate change, ecosystem quality and human health) were then endogenously integrated into the energy models of Norway and Spain. This soft-linking exercise enabled the analysis of the evolution of these life-cycle indicators, thus significantly enriching the interpretation of future energy scenarios. Emphasis is laid on the efforts made to mitigate concerns about double counting of emissions and imbalances in electricity trade processes. Overall, the interaction between life-cycle and energy systems modelling approaches is concluded to be advantageous. Nevertheless, further efforts are still required when it comes to strengthening the linkage between both approaches within a harmonised framework.

WE253
LAND USE LIFE CYCLE IMPACT ASSESSMENT: BRAZILIAN ECOSYSTEM SERVICES REGIONALIZATION
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There is a growing recognition that it is crucial to consider land use in the environmental assessment of products, especially those with a majority of their life cycle in biological processes (such as agriculture and forestry). This is an important issue for Brazil, which faces the challenge to overcome the dichotomy between production and environmental protection within the general framework of sustainable development. The present work aims to develop characterization factors (CFs) consistent with Life Cycle Assessment that estimate the impact of land use on ecosystem services for Brazil. The methodology involves four steps: i) creation of a spatial model; ii) expert consultation; iii) data collection and iv) CFs calculation. A literature review and characterization models analysis led to the choice of Brandão; Milà i Canals (2013) framework for Biotic Production Potential impact assessment as the reference to further refinement and adaptation. This work assumes that political boundaries don't reflect the dynamic nature of ecosystems, especially for countries with a large territorial area such as Brazil. Therefore, a set of criteria regarding the scientific relevance, representativity and data availability was used in order to define an appropriated biogeographical differentiation for Brazil. The results showed that the ecoregions spatial scale is a suitable choice since it synthesizes ecological, geological, botanical, climatological and hydrological patterns. Moreover, based on the criteria presented in Koellner et al. (2013) the land use classification conducted by the Brazilian Institute of Geography and Statistics was adapted for CFs calculation. In conclusion, it was possible to obtain a regionalized model for land use impact assessment with increased robustness. The next steps include consulting experts in order to identify contributions on relevance of the model assumptions and parameters, followed by the data collection and CFs calculation. Brandão, M.; Milà i Canals, L. Global characterisation factors to assess land use impacts on biotic production. **International Journal of Life Cycle Assessment**, v. 18, n. 6, p. 1243-1252, 2013. Koellner, T.; De Baan, L.; Beck, T.; Brandão, M.; Civit, B.; Goedkoop, M.; Margni, M.; Milà I Canals, L.; Müller-Wenk, R.; Weidema, B.; Wittstock, B. Principles for life cycle inventories of land use on a global scale. **International Journal of Life Cycle Assessment,** v. 18, n. 6, p. 1203-1215, 2013.

WE254
Linking water pollution to impacts on water resources
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Life Cycle Assessment (LCA) of water consumption considers impacts on the Area of Protection (AoP) "Resources", depending on the regeneration time. However, there is no method that links water pollution to the AoP resources. Because of the increasing concern over the state of water resources available for future generation, this study aims to answer if there is a link between water pollution and impacts on water resources. This talk proposes a definition of freshwater resource as part of the AoP resources and why it will be relevant to consider impacts on that AoP for certain types of pollution. A key element to clarify this debate is the concept of water functionality. The pollution in present time has potential impacts on human health and ecosystems, but beyond a certain time period persistent substances may rather contribute to potential impacts on water resources (i.e. their functionality) as it becomes increasingly uncertain to predict how the resource will be used in the future. Thus, the functionality loss due to pollution may be considered as damage to the water resource. In order to consider that link in LCIA we propose a new water use impact pathway which can be integrated into existing impact categories. Results proposed take into account the fact that it is crucial not to double-count the impacts which are already assessed on human health and ecosystem quality. For that purpose, and to fit well with the definition given to the water resource, two phenomena are considered: the persistence of pollutants in a water body and the pollution of a water resource currently unused.

WE255
Towards a new midpoint indicator for including noise impacts from mobility in life cycle assessment.
R. Meyer, LIST / Environmental Research and Innovation; E. Benetto, Luxembourg Institute of Science and Technology (LIST) / Environmental Research and Innovation; C. Lavandier, Université de Cergy-Pontoise / MRTe
Recent studies have shown that noise emissions from mobility have a significant impact on the exposed population's health. The inclusion of noise in Life Cycle Assessment (LCA) has become a necessity as noise may represent the major contributor to Human Health when the LCA study focuses on vehicles or when transports are a major part of the foreground system. Despite its importance, only a few methodological attempts to include the impacts of noise emissions in LCA have been made and a consensual approach is not available yet. According to the relevant epidemiologic literature, the work presented here considers the level of noise at which a given population is exposed as indicator. Noise emission and propagation simulation software is used to derive the exposition of a given population to noise, for the specific GIS model of the city of Lyon (France), considered as a proxy of emission, propagation and exposure conditions. The exposure of population is calculated in different geographic situations and periods of the day. The changes of exposure are then studied to understand the impact of an increment of vehicle.kilometers (vkm). A set of characterization factors (CFs), dependent of geographic characteristics and period of the day, is derived. The CFs

allow linking the inventory in vkm to a change in the population exposure. Later on, the change will then be linked to an impact on Human Health (expressed in DALYs), allowing a systematic integration of noise impact in standard LCA.

WE256

Pollinators in LCA: relevance and challenges

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Human activities are threatening biodiversity at an unprecedented scale and pace, thus potentially affecting also the provision of critical ecosystem services, including insect pollination. Insect pollinators play an essential functional role in terrestrial ecosystems, supporting ecological stability and food security worldwide. Therefore, assessing impact on pollinators is fundamental in any effort aiming at enhancing the environmental sustainability of human production and consumption, especially in the agri-food supply chains. Different drivers are leading to pollinator populations' declines. Improving a supply-chain oriented assessment of the occurrence of pressure and impacts on pollinators is needed. However, current methodologies assessing impact along supply chains, such as life cycle assessment (LCA), miss to assess impact on pollinators. In fact, none of the existing life cycle impact assessment (LCIA) models effectively accounts for pollinators. Some LCIA models have mentioned pollination, but none has presented key drivers of impact and a proposal for integrating pollinators as target group for biodiversity protection within an LCIA framework. In order to devise a pathway towards the inclusion of impact on pollinators in LCIA, we conducted a literature review of environmental and anthropogenic pressures acting on insect pollinators, potentially threatening pollination services. Based on the evidence in literature, we identified and described eight potential impact drivers, primarily deriving from industrial development and intensive agricultural practice: 1) intensified land use as a result of uncontrolled expansion of urban areas and modern agricultural practices; 2) use of pesticides; 3) presence of invasive alien plants; 4) competition with invasive alien pollinator species; 5) global and local climate change; 6) spread of pests and pathogens; 7) electro-magnetic pollution and 8) genetically modified crops. To account for these drivers in LCIA, there are specific modeling needs. Hence, the current study provide recommendation on how future research should be oriented to improve the current models and how novel indicators should be developed in order to cover the existing conceptual and methodological gaps.

WE257

Effect factors for terrestrial acidification in Brazil

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To support the increased use of existing Life Cycle Impact Assessment (LCIA) methodologies across the world, new methodological elements have been developed towards spatially resolved impact assessment. Spatially resolved methods could better capture the differences of regional environmental conditions, which is an essential approach considering countries like Brazil, with high biodiversity. Previous studies have assessed the impacts of terrestrial acidification from the estimations of the potential losses of vascular plants species richness as a result of exposure to acidifying substances for 13 biomes, with 2409 species addressed for whole world. In this context this work aims to provide spatially-differentiated effect factors (EF) for terrestrial acidification in Brazil and support the development of spatially-differentiated characterization factors for Brazil. In order to maintain compatibility with existing LCIA methods the effect factors were developed using the framework adopted by LC-Impact and Impact World+ methods. Soil pH was used as an indicator of soil acidity to predict plant occurrences. From the number of plant species occuring at each 0.1 pH unit response relationships of species richness and soil pH were developed. The species richness in each ecoregion were transformed into an empirical potentially not occurring fraction, which is a zero-to-one measure used to represent the presence or absence of species. The set of data consists of 976345 records of plants occurrences in Brazil, represented by 33167 species, indicating that this is a comprehensive study. Maps of soil pH in Brazil were extracted at 1-km resolution and pH values were extracted for the depth range of 0-30cm. For each ecoregion, species richness was plotted against soil pH and the exposure-response curves for acidification described the behavior of plant species in a certain region when it is exposed to acidic conditions. From these curves it was possible to derive the effect factors for terrestrial acidification. The results of this work show that spatial differentiation is meaningful when it is possible to combine fine spatial resolutions and highly representative data and this approach can be applied for other impact categories and regions, and contribute to the development of spatial differentiated LCIA methodologies.

WE258

A regionalised characterisation factor for freshwater and marine eutrophication applied at the territorial scale: method and application in an agricultural French catchment.

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Agriculture has impacts on the environment that range from local (eutrophication) and regional (acidification) to global (climate change) scales. To help local stakeholders manage agricultural activities, environmental impacts should be assessed at the territorial level by taking into account its biophysical characteristics. In this context, the Spatialised Territorial Life Cycle Assessment (STLCA) method was developed (Nitschelm et al. 2015). In this method, environmental impacts from agricultural territories are defined by integrating the location of emission and fate of pollutants and characterising the biophysical context. In this study, we focus on agricultural territories, which we define as territories in which most land uses or economic activities are based on agriculture. We focus here on eutrophication, because it is a major impact of agriculture at the local scale and could therefore benefit from spatial differentiation. The objectives of this study are (1) to develop two characterisation factors for freshwater and marine eutrophication, each of which considers a spatially differentiated fate for each compound responsible for eutrophication as well as a sensitivity factor of the surrounding environment to eutrophication, and (2) to apply these new characterisation factors to a case study of the "Lieue de Grève" catchment, located in northern Brittany, France. The approach developed is based on including site-specific fate factors and sensitivity at the catchment scale. For freshwater, phosphorus is considered as the limiting element for eutrophication. For marine water, we consider that nitrogen is the limiting element. Both fate factors are predicted using the Nutting-P and Nutting-N models (Dupas et al. 2015), which predict the distribution of nutrients emitted from farms into soil and water systems. Both coastal and river sensitivity indices were calculated using morphological and hydrological characteristics of river-basin and coastal areas. The method was then applied to the Lieue de Grève, which experiences eutrophication problems, and compared to a standard LCA. When using STLCA, predicted eutrophication impact was 54% lower than that using CML-IA CFs, due to differences in environmental sensitivity. Importantly, impact hotspots in the territory were identified. This study shows the relevance of using spatially explicit data when estimating local impacts in LCA.

WE259

Spatial variation of secondary inorganic PM2.5 exposure and human health impact: a case study on milk production

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Purpose: Secondary $PM_{2.5}$ human health impacts in life cycle assessment (LCA) are currently based on linear and simplified assumptions that may lead to a potential double counting. The aim of this study is to provide spatial intake fractions (iF) for secondary inorganic $PM_{2.5}$ for the U.S. and apply them to a case study that investigates environmental and nutritional effects associated with increased milk consumption. *Methods:* Unitary emissions of NH_3, NO_x, SO_2, and $PM_{2.5}$ are modelled in the Intervention Model for Air Pollution (InMAP) in three U.S. locations: Wisconsin (WI), New Jersey (NJ), and New York (NY. iFs by precursor are characterized for each emission location and are then applied to a case study investigating the environmental and nutritional health effects linked to adding one milk serving produced in WI, NJ, and NY. Environmental health impacts from global warming and particulate matter are compared to nutritional effects associated with milk consumption such as colorectal cancer, stroke, and prostate cancer using the Combined Nutritional and Environmental Life Cycle Assessment (CONE-LCA) framework. *Results and Discussion*: Intake fractions ranking is consistent between locations with $PM_{2.5}$ having the highest iFs, followed by NH_3, SO_2, and NO_x. There is a considerable spatial variation of exposure per precursor between locations due to main factors of influence. NJ shows the highest iFs followed by WI and NY. For example, the NJ estimate for NH_3 is about an order of magnitude higher than that in WI and about 15 times higher than that in NY, reflecting the higher population density. The relative ratios for NH_3/SO_2 iFs varies between locations: the ratio ranges from 18in New Jersey (abundant NO_x and SO_2 but limited NH_3) to 1.7 in Wisconsin (abundant NH_3 but limited NO_x and SO_2). Finally, adding one serving of milk to the average U.S. diet leads to an overall health benefit due to nutritional benefits if production was to occur in NY or WI while this benefit is considerably reduced when milk is produced in NJ due to substantial PM-related health effects. Conclusion: Preliminary results support a spatial variation of secondary $PM_{2.5}$ exposure in the U.S. and suggest an overestimation of health effects in regions with high NH_3 emissions or underestimation in regions limited in NH_3 from current estimates. PM and dairy related exposures and impacts are substantially greater if emissions occur in in highly populated regions limited by NH_3.

WE260

Prospects by WULCA in the impact modelling of water consumption in LCA

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Much progress has been made in the characterisation of impacts of water use and consumption in the last few years in LCA. Today, there are many generic midpoint scarcity/stress indexes (not human-, ecosystem- and resources- oriented) as well as specific endpoint-oriented methods that cover complementary cause-effect chains. Scarcity indexes are easier to use and have generally seen more application than endpoint methods, even though they rather represent competition to access water than potential impacts. On the one hand, scarcity indexes are good for water footprinting but their environmental relevance, especially for ecosystems, is limited. On the other hand, endpoint methods are ecologically relevant but they are incompatible with themselves in their current form, which prevents their integration in a single indicator. To further develop water consumption indicators in LCA, the UNEP/SETAC Life Cycle Initiative Water Use in LCA (WULCA) Working Group is currently working on the development of a framework focusing on the ecosystem quality area of protection. It adopts the generic mechanistic structure of characterisation factors used to assess emissions, with a fate factor (FF), an exposure factor and an effect factor. The FF is based on water mass balances that reproduce the hydrological cycle and the interconnections between compartments which are the base of the water cycle. It is a multimedia multipathway model that shows how the extraction of water from a compartment influences water availability in other compartments. The connection between the different water compartments helps in solving important methodological issues, such as the integration of soil moisture (i.e., green water) in the assessment of water use impacts, and the potential double counting of land use impacts on the water cycle in the land use and the water use impact categories. The exposure factor estimates the capacity of biodiversity to adapt to reduced water in a given water compartment by accessing alternative water sources. The effect factor accounts for the effects on taxa that are not able to counterbalance the lack of water. Further sub-factors, such as a severity factor, might be added to this framework. This framework clearly expands the way modelling water consumption has been considered in LCA.

Biodegradability assessments of organic substances and polymers (P)

TH001
Workshop on the current status and steps needed to improve the OECD 306 marine biodegradation screening test
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Recent ECETOC workshops have recommended a series of modifications and enhancements to existing OECD biodegradation screening tests to deliver more robust methods for assessing persistence. Specific enhancements investigated included enhanced test durations and investigating the impact of biomass density and diversity on the probability of observing biodegradability. These proposed steps were designed to minimise the high variability and poor reliability previously reported in OECD biodegradation screening tests, such as the OECD 306 marine biodegradation test, whilst increasing the ecological relevance of such studies. The Cefic-LRi funded Eco11 investigated and validated these enhancements, producing a framework for selecting the most suitable inocula cell concentration method for activated sludge (c.f. OECD 301) and marine tests (c.f. OECD 306). As a follow up during February 2015 the UK Centre for Environment, Fisheries and Aquaculture Science (Cefas) hosted an international workshop on the OECD 306 test in Lowestoft which was convened to: Initiate discussions regarding the current applications and use of the OECD 306 test; Discuss current limitations and sources of variability of the test; Provide an overview of the relevant findings and recommendations of the Eco 11 project; Discuss potential improvements and provide hands-on lab-based training of procedures used to concentrate inocula from seawater; Make recommendations for selection of test chemical and scope of a future ring test. The workshop agreed that there was too much variability in existing marine biodegradation screening tests and that there was a need, which was supported by both regulators and industry, to develop a revised method and initiate a ring test of an improved OECD 306 test. The goal is to improve the current OECD 306 marine biodegradability test to enable this to provide a more robust and effective prioritisation screen for marine persistency of chemicals used offshore or likely to enter the marine environment. A summary of the workshop discussions together with the key features of the improved test and status of the OECD 306 ring test will be presented.

TH002
Biodegradation of both chlordecone and degradation products resulting from chemical treatment
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Chlordecone (CLD: $C_{10}Cl_{10}O$) is an organochloride insecticide used to control the banana weevil from 1972. Even its withdrawal in 1993, CLD is still present in drinking water and food crop products and impacts on human health have been documented since 2001. In soils, concentrations up to 15 mg/kg have been reported which also stress its high persistency. The depollution process of In Situ Chemical Reduction (ISCR) tested in laboratory conditions by BRGM enabled to reduce by 60-70% the CLD concentration in soil. It has successfully gone through a pilot test in a banana field in martinqiue (French west Indies). This process breaks down the parent molecule in dechlorinated molecules (mainly mono, di and tri-hydro CLD), for which ones it could be hypothesized that bioaccessibility via the dechlorinated region of the molecule may be facilitated as compared to the parent molecule. We have selected various inocula, based on their historical exposure to CLD, ability to degrade other highly chlorinated compounds (PCB…), or high bacterial activity. Their ability to degrade CLD and dechlorinated CLD was tested in feed-batch systems spiked with CLD and tri-hydro-CLD, for which ones we have standards in sufficient quantity to run such tests; synthetises of purified dechlorinated CLD still being a challenge. We have a methodology based on liquid extraction and GCMS to quantify simultaneously the parent compound and several of its metabolites to ensure distinguishing loss by sorption from degradation in the feed batches with solid support on which one the highly hydrophobic compounds can sorb on. Very preliminary results after 7-month incubation did not point out a clear distinction between biodegradation kinetics for CLD and the tri-hydro CLD. We also observe a biodegradation product for CLD resulting from the opening of carbonated cage of the molecule ($C_9Cl_5H_3$), which ones biodegradation steps are still unclear. Biodegradation tests are undergoing.

TH003
Improvement of Bioavailability for Substances with low water solubility in Ready Biodegradability Tests
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Often substances fail the criterion for ready biodegradability or an unequivocal assessment is not possible because the substance is not bioavailable for the degrading bacteria due to its low water solubility. As the biodegradation of a substance strongly depends on its mass transfer and the bioavailability for the degrading bacteria, there are several technical issues which need to be addressed while testing the biodegradability of poorly water soluble substances. Due to the historic background of the guidelines for testing ready biodegradability the test concentration is relatively high (2 – 100 mg/L) and not reflecting environmental concentrations. To improve the bioavailability of poorly water soluble substances in tests for ready biodegradability the application technique and the agitation during testing need therefore special consideration. Careful investigation of the application method prior to the test start can enhance the degradation distinctly. Four techniques for the application of poorly water-soluble substances are commonly used and described in the ISO standard 10634 (1995) and recommended by the REACH guidance. These methods are direct addition, ultrasonic dispersion, adsorption onto an inert support material and the use of non-biodegradable emulsifying agents. So far no single method is recommended and routinely a combination of approaches is used for application. Based on the substance properties the application method has to be chosen carefully. These investigations involve a relatively small extra effort and often more expensive simulation studies can be avoided. The influence of different application techniques on the bioavailability and biodegradation of poorly water-soluble substances was investigated and the results will be presented.

TH004
BIODEGRADABILITY OF THE ANIONIC SURFACTANT SODIUM LAURYL ETHER SULPHATE IN INDUSTRIAL PRODUCTS
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Anionic surfactants are widely used in numerous commercial (e.g. personal care products) and industrial products (e.g. foaming agents for drilling cavities in subterranean formations). The construction of tunnels with mechanized drills produces a large amount of debris rich in foaming agents, added during the excavation process, that have an impact unknown on ecosystems. The lack of accurate information about the environmental impact of these excavated rocks has aroused increasing concern for their possible recycling as construction materials or as soil replacement for covering rocky areas. The objective of this study was to

evaluate the biodegradability of the anionic surfactant sodium lauryl ether sulfate (SLES) contained in two commercial foaming agents. For this purpose, a set of microcosms was set up using two different soils treated separately with one of the two commercial products at the concentrations used for the mechanized drill. At selected times (0, 7, 14, 21, 28 d) soil samples were collected for assessing SLES concentrationby MBAS spectrophotometric method. Moreover, microbiological analysis were performed in order to assess microbial abundance, cell viability and dehydrogenase activity. An initial negative effect on microbial abundance and viability was observed, although it was transient. At the end of the experiment SLES was no longer present in all soils and the dehydrogenase activity and cellular vitality were comparable between treated and control soil.

TH005
Effect of Different Inocula in Biodegradation Tests
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In the assessment of the biodegradability of organic substances, the role of the inoculum in a successful outcome is often overlooked. Despite ready biodegradation Test Guidelines (OECD, OPPTS) allowing the use of a variety of inoculum sources such as activated sewage sludge, sewage effluents, surface waters and soils, or a mixtures of these, typically in ready biodegradation tests a single inoculum source such as activated sewage sludge is employed. In the consideration of persistency, European Chemicals Agency (ECHA) guidance for the performance of enhanced biodegradation tests acknowledges that procedures that maximise diversity and adaptation of microbial populations in the test system are required. The work presented assesses the impact of different inoculum sources and inoculum concentration, along with the effect of mixed inoculum sources on the biodegradation of typical organic substances. Biodegradation was assessed using the OECD 301B “CO_2 Evolution Test” method referenced as EPA Fate, Transport, and Transformation Test Guidelines OPPTS 835.3110 (Paragraph (m)).

TH006
OECD 306 and Marine BODIS biodegradation tests
K. Wadhia, Opus / Environmental; S. Finn, Fjords Processing / Environmentall; R. Garrioch, Fjords Processing; J. Laud, Fjords Processing / Environmental
Each method has particular limitations which may reduce the biodegradation of materials tested or the suitability of the test method for the test substance properties. Assessment implemented will be conveyed of the suitability of the methods for biodegradation testing of water soluble organic substances and poorly water soluble / water insoluble organic substances in seawater. Unlike similar screening tests for biodegradability in freshwater systems, the marine method employs no separate bacterial inoculum, and relies upon populations of bacteria which occur naturally in seawater. The test serves only to provide a preliminary level of information on ready degradability in seawater. The overall assessment of biodegradability is based upon a comparison between experimentally determined oxygen consumption (BOD measurements) and the oxygen consumption predicted if all carbon present in the test material were completely oxidised (theoretical oxygen demand, ThOD). Where the composition of the test material is known, or can be reasonably inferred, the ThOD can be calculated from the empirical formula and the molecular weight. If neither the empirical formula nor chemical composition of the test material can be obtained, then the prediction of maximum potential BOD is obtained from the determination of the chemical oxygen demand (COD) or CHN analysis. The implications of reliance upon populations of bacteria which occur naturally in seawater rather than introduced inoculum, and the shortfalls of the procedures and scope for improvement are examined from testing and regulatory perspectives. **OECD 306, Biodegradation, BODIS**

TH007
Application of chemostat systems to include adaptation of microbial communities in persistency testing (CEFIC-LRI Eco29, University of Amsterdam)
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The aims of this CEFIC-LRI Eco29 project is to study the microbial adaptation to persistent chemicals using continuous culture systems, in order to develop guidelines for persistency tests in which the role of adaptation is accounted for. Because of its origin and history, the microbial inoculum is source of variability in biodegradability assessment. In OECD’s biodegradation testing, the short exposure times do not allow for adaptation of microbial communities to a new chemical, while in the natural environment, microorganisms tend to adapt to pollutants upon long term exposure. Which might significantly affect the results of biodegradation assessment, and might lead to the degradation of chemicals that were initially persistent. Despite numerous publications demonstrating the impact of adaptation on biodegradation test results, microbial adaptation is not include in the guidelines. Nevertheless, the importance of the adaptation phenomena in assessing biodegradability should not be underestimated. Continuous culture systems offer excellent opportunities to allow microorganisms to adapt to new chemicals under defined and environmentally relevant conditions parameters, for in theory, infinite periods of time. For our first step, environmental microbial communities (e.g. wastewater communities) are exposed to carbamazepine (CBZ) for a long term and under defined conditions, in order to follow its biodegradation and its impact on community structure and on microbial genome. Furthermore, variation of growth conditions in culture could lead to an understanding of their importance in the adaptation process. Results of this experiment will allow us to a better understanding of the relationship between microbial adaptation and biodegradation outcome and will ultimately facilitate the design of more robust screening studies and enable better predictions of biodegradation.

TH008
The impacts of light and season on isopyrazam degradation in river microcosms
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Industry uses tests provided by the Organisation of Economic Cooperation and Development (OECD) to assess the potential for transformation of a chemical and its metabolites in the environment, before it can be approved for use. Various factors are important in determining chemical fate in the environment, but many are not considered in initial laboratory tests. In particular, light can be a major influence on microbial abundance, but these tests are carried out in the dark. Additionally, microbial community abundance and diversity are likely to change in time in relation to water quality and climate, but there is little consideration of time of year that sampling takes place. We investigated the impact of light on the transformation of ^{14}C-isopyrazam, a fungicide, in river microcosms, using water and sediment collected at defined seasonal time points across an 18-month period. Materials were collected from the River Dene (Wellesbourne, UK), and both water and water-sediment microcosms were pre-incubated in the dark or light (18 hour light/6 hour dark cycle) for 9 days prior to isopyrazam addition (0.1 mg/L). There was little degradation of isopyrazam in dark microcosms regardless of season. However, in microcosms incubated under a light-dark cycle, degradation was faster in all seasons, although the rate of degradation varied depending on time of year sampling took place. Microcosms sampled in autumn or summer gave the fastest degradation rates, whereas microcosms containing samples collected in winter gave the slowest degradation rate. This trend was seen in both the water and water-sediment microcosms, although there was a greater effect in water-sediment systems. There were also differing degrees of mineralization across time, but the extent of mineralization was not correlated with the overall isopyrazam degradation rate. To date, microbial community analysis has focused on phototrophic communities using chlorophyll *a* abundance as a measure. While phototrophs appear to be important determinants of isopyrazam degradation, overall abundance of phototroph communities was not closely related to differences in degradation of isopyrazam across time. This suggests that phototroph community composition, or interactions between phototrophs and other taxa, could underlie temporal variations in isopyrazam degradation.

TH009
Multiple use of vegetation in dioxin contaminated soil
J. Wittebol, M.H. Wagelmans, E. Elferink, Bioclear
There is an ongoing global and socially relevant transition towards biobased production processes. Biobased production processes use renewable resources from the living environment (biomass, green materials) as part of the sustainable economy. Due to long term and widespread use of POP’s for example PCB’s, PAH or dioxins soils can be polluted with these chemicals. This might result in pollution of renewable resources. The sugar beet for example is a versatile product. It is used to produce sugars, molasses and pulp. These sugars are sequentially used in foods, biofuel or PET + PEF bottles. PAH is detected in molasses and dried sugar beet pulp. Dioxins, PAH and PCB’s are lipophilic organic compounds and are more prone to absorb to the root or soil particles than being taken up by root into the inner root and for transport into the rest of the plant. There are however exceptions. The goal of this research is to investigate possibilities to connect the biobased production with the removal strategy of POPs from soil. The possibility of composting PCDD, PCB’s and PAH contaminated biomass was investigated in both aerobic as well as anaerobic digested systems. When organic waste streams are contaminated with POP’s the toxicity might be affected by additional processes for example biological degradation. This could either be positive or affect the process negatively. And thus the application of contaminated waste should be considered in detail. With the application of the (possibly) contaminated renewable resources the value of the agricultural land as well as the “agricultural waste” can be increased. It is unknown if biorefinery production processes are affected by persistent contaminants like dioxins. The main question is which processes occur both during uptake and during biobased refinery. What does this mean for the final product and possible waste streams? And can “pollution” of the end product be prevented by additional processes in soil and during refinery?

TH010
Risks of Non Extractable Residues (NER) of chemicals in soil
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Institute for Molecular Biology and Applied Ecology / Ecological chemistry; K. Hund-Rinke, Fraunhofer IME; J. Lahr, Alterra; J.W. Deneer, Alterra Wageningen UR / ERA team

With regard to regulation of chemicals, Non Extractable Residues (NER) are considered as an increasingly important issue. Depending on the stakeholders, NER is considered in two ways, either as a chemical time bomb or as a sustainable way of detoxification. ECETOC has provided a proper basis for our study with ideas on extraction regimes and tests to access the risks. In our study we investigate three substances that are known to give a Non Extractable Residue; TNT, Cypermethrin and Carbendazim. In our chemical approach we distinguish the measurable parameter Total concentration (harsh extraction) and the bioavailable concentration as can be measured with Tenax or in the water phase. Bioassays are used two follow the change in toxicity during aging and the probable formation of NER. By selective extraction of the bioavailable fraction, toxicity should decrease. The investigation of the validity of this assumption is an important part of our study. It is not possible to measure NER under normal conditions. Radiolabelled versions of the chemicals are necessary to establish the fate of the chemical. Residual radioactivity after extraction is considered to be the result of formation of NER. This can be caused by a very strong binding of the chemical, but also by inclusion of the radiolabel in the biomass or carbonates or inclusion of intermediates of degradation. In our study we have followed radioactivity in the spiked and aged soils using three different and representative soils Our study aims to provide a clear, mechanistically driven definition for NER and residual toxicity caused by NER. As stated by Ortega-Calvo et al. (2015) this should be measurable in order to have a clear function in regulation. For this purpose we develop a “soup test” to assess the toxicity of the different fractions of chemicals.

TH011

Ready biodegradability of poorly water-soluble substances: an evaluation strategy proposal

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Biodegradability is a key parameter in several international regulations on chemical substances, such as the European Union REACH and CLP regulations. In order to evaluate the complete mineralisation of chemicals, i.e. ultimate biodegradation, tests have been designed under the umbrella of international organisations such as the OECD (Organisation for Economic Co-operation and Development) and ISO (International Organisation for Standardisation). Among these tests, the ready biodegradability tests (RBTs) enable an assessment of the ultimate biodegradability in aqueous media under aerobic conditions, but only few guidance documents have suggested technical adaptations to improve the bioavailability of poorly water-soluble chemicals. In this context, the aim of this study is to develop a two-phase evaluation strategy to select the most relevant Bioavailability Improvement Methods (BIMs) for enhancing the biodegradability of tested substances. Tests were performed with a solid (Anthraquinone) and a liquid (Isodecyl Neopentanoate) substance versus 5 BIMs: i) ultrasonic dispersion, (ii) dispersion using an emulsifier, (iii) adsorption onto silica gel, (iv) dispersion with silicone oil, (v) dispersion with an emulsifier and silicone oil. The calculation of a BIM classification index is proposed, which enables the ranking of the different operating conditions.

TH012

New biodegradation tests for chemical compounds at low environmental relevant concentrations

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Existing biodegradation methods such as OECD tests are typically carried out at relatively high substrate concentrations such as 20 mgC/L that may result in under- or over- estimation of observed substrate utilization kinetics at environmentally relevant low concentrations. Furthermore, although well established, OECD guidelines are largely not suitable for testing hydrophobic and volatile compounds such as fragrances. Our main goal is to develop a standardized and validated growth-linked biodegradation test as an alternative method to existing biodegradation tests? in the range of 0.1 - 10 mgC/L. Our methodological concept is based on comparative evaluation of cell density measurement by flow cytometer and substrate disappearance measurement by CO2 evolution and gas chromatography under assimilable organic carbon restricted conditions. We conduct our experiments with lake water microbial communities at starting cell density of 10E4 and 10E5 cells/mL in a defined mineral medium. Sodium benzoate, 1-octanol, anthraquinone and phenol are selected as primary positive controls for readily biodegradable compounds. We observed step-wise increase in the lake community size (at initial cell density of 10E4 cells/mL) at the expense of added positive control substance at concentrations of between 0.1 – 2 mgC/L and 1–2 mgC/L, respectively. Yield approximations from the observed community growth was in line with CO2 evolution test results for the target compounds. For accurate mass balance between compound and community size, we will further simultaneously measure lake water community growth and compound disappearance by gas chromatography. Lake water communities that enable biodegradation of the test compounds will be analyzed for diversity changes, and we will also further isolate and characterize degrader bacteria. **References:** Czechowska K, Sentchilo V, Beggah S, Rey S, Seyfried M, van der Meer JR. (2013). Examining Chemical Compound Biodegradation at Low Concentrations through Bacterial Cell Proliferation. Environ Sci Technol. DOI: 10.1021/es303592c

TH013

Coupling traditional biodegradation assessments with spectroscopic tools for plastic wastes: case of Polyurethane wastes assessment

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Development of new strategies to assess chemicals fate is needed. Among the diverse family of chemicals, synthetic plastic wastes are of great concerns due to their robust accumulation in landfills, which is estimated to account for 10 to 30% (w/v) of total wastes in landfills (in USA and in Japan). Strategies to assess the biodegradability of these polymers are generally complex and limited in their efficiency, because they are confronted to a mixture of chemicals having each one their own intrinsic properties in terms of solubility, toxicity and persistence. To assess the biodegradability of a polyurethane waste generated by an industrial, we’ve attempted to develop a strategy based on traditional assessments for toxicity and biodegradation (carbon balance) coupled with the Raman spectroscopy to evaluate both quantitatively and qualitatively changes occurring during the polymer waste biodegradation. The carbon balance analysis enables to identify polyurethane compartmentalisation after the microbial biodegradation: 0.5% of the carbon has been converted in biomass, 3.9% was released as inorganic carbon and 23.2% as volatile organic carbon. The majority of the carbon (72.5%) remains as polyurethane powder after the biodegradation event. To evaluate the impact of the microbial inoculum onto the chemical structure of the polyurethane, spectral analyses were done. Some major changes have been operated in the chemical structure of the polymer as the disappearance of certain peaks corresponding to ether (1,181 cm^{-1}) and aromatic C-C bonds (1,520 cm^{-1}). According to the untreated polyurethane analyses, it appears that the polymer structure is deeply altered and that its polyether fraction has been degraded by the inoculum. To conclude, coupling the traditional assessments methods and the spectroscopic profiling enabled to characterise how the polymer is affected by the inoculum. Although the polyurethane was degraded only at 27.5%, a deep change in its chemical structure was operated leading notably to the disappearance of some rich region composed of high content of polyethers. This assessment strategy appears to be relevant to better understand in a quantitative but also in a qualitative manner how the synthetic plastics can be altered by microorganisms. Keywords: Biodegradation, Polyurethane biodegradation, Raman spectroscopy, carbon balance, inoculum adaptation

Exploring links between the biodegradation of chemical contaminants, the metabolic capability of microbial communities and environmental variables (P)

TH014

Effects of agricultural practices on organic matter degradation in ditches

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Detritus processing relies on the quantity and quality of dead organic matter available as food for the detritivorous community and is essential to global carbon and nutrient cycling. Agricultural practices have direct adverse effects on biodiversity, but also can differentially affect the input of terrestrial organic matter (OM) sources and agricultural chemicals into adjacent aquatic ecosystems. Hence, agricultural practices potentially affect OM quality and inherent decomposition by microorganisms and consumption by invertebrates in adjacent agricultural drainage ditches. This study therefore aimed to determine the effect of agricultural practices on OM degradation by microorganisms and invertebrates in agricultural drainage ditches. A consumption and food preference experiment was performed in the field and in the laboratory using natural OM collected from the field that reflects the agricultural practices ‘dairy farm grasslands’ and ‘hyacinth bulb fields’, in which freshly cut grass and hyacinths were also offered to control for OM composition. Large- and small mesh-sizes were used to distinguish microbial decomposition and invertebrate consumption. Results showed that OM collected from ditches adjacent to grasslands and freshly cut grass and hyacinths were preferred over OM collected from ditches adjacent to hyacinth bulb field, in which both microbial decomposition and invertebrate consumption were strongly retarded. This is likely the result of sorption and accumulation of pesticides, rendering sediment OM unsuitable for microbial decomposition and invertebrate consumption. This outcome illustrates that differences in agricultural practices can, in addition to direct detrimental effects on aquatic organisms, indirectly alter the functioning of adjacent aquatic ecosystems.

TH015

Fullerol as a potential pathway for mineralisation of fullerene nanoparticles in biosolids-amended soils

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Fullerenes (e.g. $C_{60,}C_{70}$) nanomaterials are now used in a wide range of products and thus can enter the environment via waste streams. Although these contaminants could reach and potentially adversely affect the terrestrial environment via the re-use of biosolids, their fate in soil is poorly understood. This study examined the mineralisation of C_{60} and its transformation product fullerol (C_{60}-OH with drastically different physico-chemical properties) in biosolids-amended soils. Using radiolabelled C_{60} and C_{60}-OH, we established that mineralisation of C_{60} was less than the detection limit (< 0.025%) in three contrasting soils during a 55 day long experiment. In contrast, however, up to 3% of C_{60}-OH was mineralised during this period. This difference in observed mineralisation highlights C_{60}-OH's greater bioavailability compared to its parent compound, C_{60}, in biosolids-amended soils. These results indicate that transformation of certain fullerenes, such as to fullerol, could provide a potential pathway for their mineralisation, but such transformation may be slow. Glucose-induced respiration tests on C_{60}-/ C_{60}-OH contaminated soils suggested that overall microbial activity was not compromised by the contamination. This study is also the first work to examine the fate of fullerol in soils.

TH016

Kinetics of rapid covalent bond formation of aniline with humic acid: ESR investigation with nitroxide spin probes

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The bioavailability of many soil and sediment contaminants depends on their interaction with the organic matrix. In biodegradation simulation studies, many of these xenobiotic chemicals exhibit a large fraction of residues, which cannot be further extracted even by harsh methods (non-extractable residues, NER). The paper presents a new approach of using stable paramagnetic spin probes for investigating the kinetics of covalent binding of xenobiotic functional groups with humic acids, the main organic matter fraction. Leonardite humic acid (LHA) was incubated with the nitroxide spin label Anilino-NO (2,5,5-Trimethyl-2-(3-aminophenyl)pyrrolidin-1-oxyl), which includes an aniline functionality susceptible to interaction with LHA. ESR spectra of LHA samples were recorded at X-band frequency (9.43 GHz) at room temperature. A broadened ESR signal was observed of Anilino-NO incubated with LHA, which indicates strong restriction of the reorientational motion of the spin probe, i.e. immobilzation due covalent binding of the aniline group. This signal increased immediately after incubation and was used to determine the kinetics of the covalent bond formation. A two-sites model with two parallel first-order reactions was used for fitting the measured values and determining the rate constants. A fast reaction with a rate constant of 0.308 min^{-1}, i.e. a half-life of approximately 2.25 min, and a slower reaction with a rate constant of 0.012 min^{-1}, i.e. a half-life of 57.8 min, was observed. Laccase-treated LHA shows an increase of the amount of reactive sites, presumably quinones, by a factor of 7.2 for the slow reaction, which supports the conclusion of a nucleophilic addition reaction of the aniline moiety with LHA. Both reaction rate constants are only marginally enhanced for the laccase-treated LHA. Paramagnetic spin probing is an effective method to investigate the rapid formation of covalent binding of xenobiotic functional groups humic acids. For chemicals containing an aromatic amino group a considerable fraction of NER can be explained as covalently bound residues, which is important for the determination of persistence in soil and sediment required for PBT and risk assessment of pesticides, biocides and other chemicals.

TH017

Fate, biodegradation and mineralization of two labeled pesticides in soils under different environmental conditions

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Objectives of the Study To study the turnover mass balance (CO_2 evolution, extractable contaminant residues and non-extractable residues) of ^{13}C-labeled 2,4-D and ^{13}C, ^{15}N-labeled Glyphosate in soils under different environmental conditions. To investigate the incorporation of ^{13}C-label into microbial fatty acids, and $^{13}C^{15}N$-labels into amino acids from ^{13}C-labeled 2,4-D and $^{13}C^{15}N$-labeled Glyphosate in soils. To quantify the extent of "biogenic residues" formation during the degradation of two pesticides under different environmental conditions. To define how the environmental conditions (e.g. temperature, soil organic matter and soil pH) the biodegradation of pesticides and the extent of "biogenic residues" formation. **Material and Methods** Soil samples were collected from the 0 to 5 cm soil layer in the agricultural field located in Bad Lauchstädt, Germany. The soil organic matter (OM) of the soil and the pH were modified reaching 3%OM and 4%OM and pH 5.5 and 6.0, accordingly. Thereafter, soil was spiked with a labeled pesticide (^{15}N-$^{13}C_3$-Glyphosate or $^{13}C_6$-2,4-D) and incubated according to the OECD 307 and at different temperatures (10°C, 20°C and 30°C). The $^{13}CO_2$ evolution was quantified by Inorganic Carbon Analyzer and the isotopic composition was measured by GC-IRMS. After the incubation, the residual parental pesticide + its primary metabolites and microbial biomass components (amino acids) were extracted and quantified and identified using UHPLC (Glyphosate) or GC-MS (2,4-D, amino acids) and the isotopic composition was measured by GC-IRMS. **Results** After 39 days of incubation, mineralization of $^{13}C_3$-glyphosate in soil containing 3%OM, 4%OM and pH 6 at 10°C, 20°C and 30°C was higher than 20%, 45% and 50%, respectively. In the same experiment with soil at pH 6 and pH 5.5 at 10°C, 20°C and 30°C the mineralization of glyphosate was lower and reached about 16%, 35% and 40% of $^{13}C_3$-glyphosate equivalents, respectively. Mineralization of $^{13}C_6$-2,4-D in soil containing 3%OM after 32 days of incubation constituted more than 18%, 50% and 70% of $^{13}C_6$-2,4-D equivalents at 10°C, 20°C and 30°C, respectively. In the soil at lower pH (5.5 and 6), mineralization of this herbicide was lower and reached accordingly 2%, 20% and 20% at 10°C, 20°C and 30°C. **Conclusion** The first results showed a positive influence of the environmental conditions like temperature and OM content of soil on the extent of glyphosate and 2,4-D mineralization in soil.

TH018

Identification of biotransformation products of the herbicide Imazapic via suspect screening

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Imidazolinones are selective herbicides used in weed control of agricultural areas. These substances persist in soils with half-lives of up to 233 days and some of these compounds have been prohibited in European countries. While a number of studies focused on their ready photochemical transformation, far less is known about their microbially mediated transformation pathways in aquatic systems. Compared to the clean matrix of aqueous photolysis experiments, the identification of biotransformation products is much more challenging. The present study tested the susceptibility of the imidazolinone imazapic (IMA) to microbial transformation in order to elucidate the formed transformation products. An activated sludge inoculum was used for spiked batch experiments and regularly fed with additional primary substrate. After a total runtime of eight days, 23 % of IMA was transformed. In a first step, we screened samples for 90 previously defined suspected transformation products using LC-Q/TOF accurate mass scans. Six suspect transformation products could be matched by their precursor *m/z* with a mass accuracy of < 2 ppm. Subsequently, MS/MS spectra were recorded and interpreted to confirm the suspected compounds. Further structural product ion elucidation also allowed for indication of the transformation site on the molecule. In this way, decarboxylated-IMA, hydroxylated-IMA, demethylated-IMA and *N*/*O*-methylated-IMA could be identified as biotransformation products. The detected TP293 is tentatively assigned to a product hydroxylated at a double bond (elemental mass shift of H2O), possibly a ring opening reaction. A further product, TP276, indicated NH substitution by an OH moiety, but requires further confirmation. The results indicate preferential microbially mediated transformation reactions at specific sites on the molecule, which can be further used as model for other imidazolinones with similar structures.

TH019

Biodegradation of synthetic β-triketone herbicides

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Agricultural use of pesticides ensures a higher crop quality and production but it is also one of the major sources of diffuse pollution in the environment. Microbial degradation is considered as an important dissipation process limiting the accumulation of pesticides in the environment. In this context, two bacterial strains able to degrade sulcotrione, a β-triketone herbicide, were isolated from an agricultural soil previously exposed to this herbicide. The two isolates were identified using 16S rRNA gene sequencing as *Pseudomonas sp.* 1OP and *Bradyrhizobium sp.* SR1. Their capacity to degrade aerobically sulcotrione was estimated and 2-Chloro-4-mesylbenzoic acid (CMBA), one of its main metabolites, was clearly detected. Their ability to degrade other β-triketone herbicides was tested showing that only *Bradyrhizobium sp.* SR1 was able to completely degrade another β-triketone herbicide, mesotrione and producing already known metabolites (AMBA and MNBA). Microbial toxicity of sulcotrione and mesotrione and their related metabolites in bacterial cultures was estimated by monitoring 4-hydroxyphenylpyruvate dioxygenase (HPPD) enzyme inhibition. Our results indicate that β-triketone herbicides toxicity linked to HPPD inhibition was due to parent molecules, and not to the formed metabolites. Attempts were done to identify the genetic localization of sulcotrione degradation in *Pseudomonas sp*. 1OP and *Bradyrhizobium sp*. SR1. Plasmid profiles of both strains revealed the presence of large plasmids (>12 kb and >50 kb, re*sp*ectively). Curing experiments showed that *Pseudomonas sp*. 1OP lost its ability to degrade sulcotrione under non-selective conditions, therefore degradation capacity may be attributed to the presence of this plasmid. On the contrary, under the same conditions, *Bradyrhizobium sp*. SR1 plasmid was not cured and the sulcotrione-degrading ability of the strain was maintained. Furthermore, a 14 000 Tn*5* mutant library was constructed using a Tn*5* mutagenesis approach conducted on *Bradyrhizobium sp*. SR1. Among this library, two mutants affected in their biodegradation capacity were identified. Full sequencing of SR1 and Tn*5* mutants is ongoing to identify possible degrading gene candidates. Keywords: biodegradation, β-triketone, herbicides, metabolites.

TH020

Fingerprinting Micropollutant Transformation in Hyporheic Zones

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Hyporheic zones are key compartments for the functioning of aquatic ecosystems. As dynamic and complex transition regions between rivers and aquifers, they are characterized by the simultaneous occurrence of multiple physical, biological and chemical processes. Assessing persistence of environmental contaminants in the hyporheic zone is non-trivial. In addition to advection processes, transformation can be influenced by numerous biogeochemical factors, including physical characteristics of the sediments (e.g. porosity and bed morphology), chemical parameters (e.g. occurrence of electron donors or acceptors), and biological factors (number and type of microbial population). These variables can lead to difficulties in reproducing lab-based biodegradation experiments and in extrapolating estimates of persistence in the lab to field conditions. Benchmark chemicals (i.e. model substances which are co-incubated with a substance of interest in lab-based biodegradation experiments) have been proposed as a means of addressing inter-assay variability in sediment biodegradation experiments. These controls may aid in extrapolating amongst various lab-based experimental designs and conditions, and perhaps even to conditions encountered in the field. The present work takes an initial step towards characterizing a suite of benchmarking chemicals for use with sediment biodegradation experiments by measuring their degradation half-lives ($DegT_{50}$) under a series of carefully controlled conditions (temperature, oxygen content, stirring) in closed-bottle experiments. Natural sediments from the river Erpe (Berlin, Germany) were collected and characterized prior to use. Marker chemicals (which included pharmaceuticals and industrial chemicals) were selected based on their environmental occurrence and to cover a range of physicochemical properties, transformation rates and pathways. We expect this approach to aid in the development of more appropriate assays specifically designed for probing micropollutant transformation in the hyporheic zones, for extrapolation of micropollutant half-lives between lab and field, as well as among diverse river sediments and within various locations within a river system.

TH021

The role of solar radiation on the photodegradation of PAHs in soils

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Polycyclic aromatic hydrocarbons (PAHs) are a family of widespread environmental pollutants, whose chemical structure is based on two or more fused benzene rings. PAHs are released from natural and anthropogenic combustion processes. Although these chemicals are mostly released to air, soil is considered as one of the major sinks of atmospheric PAHs, undergoing wet and dry deposition processes. Since the environmental fate and transport of PAHs is highly influenced by temperature and solar radiation, photodegradation is likely to be the main process related to the loss of PAHs on soil surfaces, leading to the potential formation of more toxic metabolites than their parent compounds. This study was aimed at estimating the photodegradation rate of PAHs in soils under winter conditions in the Mediterranean region. Soil samples were taken from the A horizon of two common Mediterranean soils located in remote areas. Arenosol soil samples corresponded to Haplic Arenosol, an acidic and coarse-textured soil with granitic origin. In turn, Regosol soil samples belonged to Calcaric Regosol, which is formed of sedimentary materials and fine-textured soil. Ten grams of cleaned soil were deployed in uncovered glass Petri dishes. Every sample was spiked with 250 μL of a stock solution containing 16 US EPA priority PAHs at a concentration of 100 μg/mL and exposed to sunlight radiation in a methacrylate box placed on the roof of the School of Chemical Engineering, Tarragona, Catalonia. Dark controls were also performed to assess slow sorption processes. Temperature and solar radiation intensity were simultaneously monitored over the experiment. The results showed that PAHs behaved differently according to their molecular weight, temperature, radiation and soil texture. A decreasing trend of PAH concentrations in both soils in samples exposed to daylight was observed. Low molecular weight PAHs are more influenced by volatilization and sorption, while photodegradation is more evident for medium and high molecular weight PAHs. Photodegradation rates were higher than those obtained previously in laboratory conditions, since the intensity of solar radiation is ten times higher than that emitted by fluorescents in a climate chamber.

TH022

Bioavailability-related effects of dissolved organic matter on biodegradation of PAHs

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In environmental sciences, dissolved organic matter (DOM) is usually differentiated from particulate organic matter as the size fraction of organic matter smaller than 0.45 μm. This fraction typically consists of a multitude of structurally different compounds, all typically present at low concentrations, although it can differ significantly in quality (or biodegradability) and quantity in time and space. The environmental fate of organic pollutants, such as PAHs, can be affected by DOM via increased apparent solubility, desorption, transport and biodegradation. In our group, we have specifically addressed the influences of DOM quality on bioavailability-related phenomena: chemotaxis, attachment and solubilisation. We used, for our studies, different experimental models to assess the bioavailability of PAHs for biodegradation, and these included Tenax extraction, dual 14C/residue analysis of microcosm samples, dynamic passive dosing with PDMS, biphasic NAPL/water systems, and column systems. Different model DOM sources, of dissimilar quality but all with potential use in bioremediation were used, and included humic acids, root exudates, biosurfactants and organic fertilizers. We found that biodegradation of poorly bioavailable PAHs was enhanced by (bio)surfactants (Environ. Sci. Technol. 48:10869-10877, 2014), the targeted fertilization of free-oil phases or NAPLs (Environ. Sci. Technol. 45:1074-1081, 2011), by modulating the deposition and tactic motility of microbial degraders in porous media (Environ. Sci. Technol. 46:6790-6797, 2012), and by root exudates (Soil Biol. Biochem. 57:830-840, 2013; Environ. Sci. Technol. 49:4498–4505, 2015). However, a negative influence on biodegradation of PAHs by humic acids (Environ. Pollut. 184:435-442, 2014) and biosurfactants (Environ. Pollut. 205:378-384, 2015) was found if they prevented cell attachment to the PAH-loaded PDMS sources. These influences of DOM on bioavailability are relevant not only for innovation efforts in bioremediation but they have also connections with the determination of bioavailability of organic chemicals in retrospective and prospective risk assessment and regulation (Environ. Sci. Technol. 49:10255-10264, 2015).

TH023

Investigation of motor oil biodegradation by different bacterial strains

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Large amount of motor oil is used in many branches of industry. The new generation of motor oils contains high percentages hydrocarbons (C16-C36), more than 75% cycloalkane and polycyclic aromatic hydrocarbons (PAH). However, used motor oils contain a higher percentage of aliphatic and aromatic hydrocarbons, nitrogen and sulfur compounds, and metals (Mg, Ca, Zn, Pb, etc.). In addition to these compounds, the presence of PAHs (naphthalene, benzo [a] pyrene and anthracene) is also expected. Therefore these oils represent a great danger to the environment, because all these compounds are known as mutagenic and carcinogenic [1, 2]. Globally, biodegradation is a common choice for remediation, because the pollutants become substrates for the growth of microorganisms [3]. The aim of this experiment was to investigate the biodegradation of motor oil by bacteria *Stenotrophomonas sp*. (NR 1), *Rhodococcus sp*. (UG 10) and *Bacillus sp*. (F 231) isolated from activated sludge ofindustrial wastewater treatment plant. The process of biodegradation of motor oil was monitored for 45 days. Each 15 days the process was stopped, the hydrocarbons were extracted, and the samples were analyzed by gas chromatography. Comparison of motor oil concentration in the samples and in the abiotic controls provided insight into the biodegradation activity of bacteria. The results showed a reduction in concentration of motor oil comparing to the beginning of the experiment (300 ppm). The growth of bacteria confirmed that the motor oil was the only source of carbon. The reduction of motor oil concentration was correlated with the number of bacterial cells. Based on these results, it was concluded that the biodegradation activity was highest in *Bacillus sp.* (F 231). After 45 days this bacterial strain degraded 95.4% of motor oil. Under the same conditions two other bacterial strains showed lower biodegradation activity: 84.9% by *Stenotrophomonas sp.* (NR 1), and 76.6% by *Rhodococcus sp.* (UG 10). Keywords: biodegradation, motor oil, bacterial stains References: [1] Bhat, M.M., Shankar, S., Shikha, Yunus, M., Shukla, R.N., *Adv. Appl. Sci. Res.* **2** (2011) 321-326. [2] Obayori, O.S., Salam, L.B., Ogunwumi, O.S., *J. Bioremed. Biodeg.* **5** (2014), 1-7. [3] Anastas P., Eghbali N. *Chem. Soc. Rev.*, **39** (2010), 301-312.

TH024

Bacterial community structure and biogeochemical activity in an aquifer contaminated with pesticides

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Our objective was to assess the effect of cocktails of pesticides on groundwater microbial abundance, community structure and their nitrate reducing activity. We used two complimentary approaches: a 2-year *in situ* monitoring at the Ariege alluvial plain (France) and microcosms with groundwater with contrasted contamination history spiked with selected herbicides having a high occurrence in this aquifer, atrazine (ATZ), desethylated atrazine (DEA) and ATZ+DEA. Abundance of the universal marker (16S rRNA) and of nitrate-reducing bacteria (*nar*G and *nap*A) was assessed by qPCR. Presence of the ammonium oxidizer was monitored by the *amoA* gene by PCR. Biodiversity was assessed using a fingerprinting technic (CE-SSCP). Pesticides in water were analyzed by LC-MS/MS. In microcosms, biodiversity was higher in historically contaminated water than in pristine-like one and remained higher under laboratory incubations. Biodiversity was significantly affected by both the chemical concentration and the incubation duration (not the chemical type) in the pritine-like water while in historically contaminated water, it was affected by the incubation duration only (not the chemical type or concentration), suggesting a community tolerance to the pesticides induced by chronic exposure. Biomass and denitrification gene abundance was in most cases higher at 10 μg/L than at 1 μg/L or in control, especially at 30-d incubation in both water types. During the two-year *in situ* monitoring, tendencies between chemical and microbial criteria were similar to the microcosm outcomes however they did not result in significant relationships. More specifically, it was not observed a significant relationship between nitrate concentration and microbial biomass and abundance of denitrifying genes (*narG* and *napA*). Microbial end points based on molecular indicators should be proposed to complete the biodiversity objective under the European water directive framework with the microbial compartment.

TH025

Analysis of the microbial community of the river Tiber in different contamination points along its course

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One of the major issues in freshwater quality assessment is finding a link between the chemical and ecological status of a water system. Filling this gap is a new challenge for finding new biological indicators, by integrating multiple stressors, to describe/assess water quality thoroughly. In this context, the main aim of the MicroCokit FP7-PEOPLE-2012-IAPP Project is to identify microbial community based indicators for monitoring and evaluating different types of anthropogenic and environmental pressures. The case study is the river Tiber in which four different sampling sites along its course have been chosen and sampled in Autumn and Spring for 2 consecutive years. Except the pristine river source, the other three selected sites were differently exposed to anthropogenic pressures: Agricultural, Industrial and Anthropogenic one, respectively. Chemical analysis (inorganic elements, DOC, PAHs, organochlorine, triazine, chloroacetamide pesticides, perfluorinated compounds, pharmaceuticals, etc.) have been performed, together with the phylogenetic analysis of the bacterial community by Fluorescence In Situ Hybridization (FISH). Overall results of four sampling campaigns show how the changes in the microbial community structure reflect both natural environmental variations such as river course and seasonality, and the different sources of contamination.

TH026

Microbial turnover of PAH: analysis of degradation and dissolution kinetics and simulation of remediation options

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Polycyclic aromatic hydrocarbons (PAH) are hydrophobic compounds exhibiting high toxicity and carcinogenicity. Originating from tar, coking and incomplete burning processes, PAH have contaminated many industrial areas and most urban soils. Feasible strategies for minimizing related adverse effects include the utilization of microbial degradation processes, where hardly soluble compounds like PAH pose a particular challenge. For the prospective assessment of the turnover of PAH there is a research gap since only very limited kinetic data for different groups of PAH degrader bacteria are available. In particular for Mycobacteria the knowledge is limited due to the complex cell cycle with formation of cell clusters and aggregates with PAH. From experiments and inverse modelling, growth and affinity parameters have been determined and compared for well described phenanthrene and pyrene degrading Mycobacteria on both substrates. The PAH were present as microcrystals in suspension. We refined a recently developed numerical model for desorption and metabolism, taking simultaneously into consideration chemical activity, sorption and dissolution processes, metabolism and growth as well as cell maintenance and decay in non-steady-state. This model was applied to prospectively describe PAH turnover dynamics for various treatment options to remediate contaminated soils. The investigated Mycobacterium species were not superior in PHE degradation to strains investigated earlier with this method. The amount of PAH ultimately degraded rather depended on the ad/desorption rates, and hereby on the substrate flux to the microbes, than on the Monod and Michaelis-Menten parameters of the strains. Predictive simulations revealed that bioaugmentation would only have a small and short-term effect on biodegradation. The addition of adsorbing soil amendments would shift the remaining PAH to the adsorbed/sequestered pool leading to a decrease in toxicity (lower mobility and bioavailability), but also to a lower diffusive flux to microbial cells and thus to declining activity of the PAH degrading microbes. Mobilization of PAH by adding solvents or surfactants would foster microbial activity, but might also increase toxicity. As the most promising strategy, stimulating cometabolism (e.g. by adding compost) could maintain microbial mass and activity at a high level leading to a steady decline of PAH in all pools. The model can thus be a valuable tool for assessing remediation options.

TH027

enviPath - The environmental contaminant biotransformation pathway resource

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The University of Minnesota Biocatalysis/Biodegradation Database and Pathway Prediction System (UMBBD/PPS) have been a unique resource covering microbial biotransformation pathways of primarily xenobiotic chemicals for over 15 years. This poster will introduce the successor system, enviPath (The Environmental Contaminant Biotransformation Pathway Resource), which is a complete redesign and reimplementation of UM-BBD/ PPS. enviPath uses the database from the UM-BBD/ PPS as a basis, extends the use of this database, and allows users to include their own biotransformation pathway data to support multiple use cases. As one new use case, we will present the package "DAR soil", which contains pathway information from soil degradation studies. This information has been extracted from pesticide registration dossiers (draft assessment reports, DAR) made publically available through the European Food Safety Authority. The package also contains information on different experimental conditions, which are stored as "scenarios". Compounds in the pathway are associated with a given scenario if they have been observed under the specific experimental conditions. If available, a biotransformation half-life (DT50) value is associated with a given compound in the pathway and a specific scenario. Much like the UM-PPS system, enviPath further provides different models to predict likely biotransformation pathways based on biotransformation rules. It supports relative reasoning for the refinement of predictions and allows its extensions in terms of previously published, but not implemented machine learning models. User access is simplified by providing a REST API that simplifies the inclusion of enviPath into existing workflows. An RDF database is used to enable simple integration with other databases. enviPath is publicly available at https://envipath.org with free and open access to its core data.

TH028

Evaluating and validating a Quantitative Structure Biodegradation Relationship (QSBR) model with experimentally determined biodegradation rates

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Biodegradation is a principal fate process of organic chemicals in the environment that affects regulatory persistence assessments and the design and operation of effective biological wastewater treatment systems to protect the aqueous environment. The rate of biodegradation is particularly difficult to predict as it is influenced by many different factors. These include inherent ones, such as the chemical structure, and external ones, such as the probability of encountering a bacterium able to degrade the chemical in a given environmental compartment. The systematic investigation of these factors has received little attention. In this study, we have so far developed a set of quantitative structure biodegradation relationships (QSBRs) for diverse set of aromatic micro-pollutants, which relate biodegradation rates (estimated from EPISuite) to different molecular descriptors. Such rates are derived from several biodegradation databases, which often lack accurate quantitative information for reliable determination of the rates. To experimentally validate one of the models, we have therefore selected several representative chemicals to perform biodegradation experiments using bacteria with known degradation pathways. A complete mass balance of each chemical was conducted by measuring the chemical concentration, microbial growth and carbon dioxide during the experiment. Finally, the biodegradation rate of the selected chemicals was estimated using these data and compared against the molecular descriptors from our previously developed model to test the robustness of its predictions.

Mercury Biogeochemistry and Policy (P)

TH029

Relationship between Hg species in seawater and microbial communities in the Middle Adriatic Sea

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Chemical and biologically mediated reactions can transform mercury species in the water column. These reactions can facilitate mercury's entrance into the marine food web in which it bioaccumulates in the form of methylmercury (MeHg). Factors that promote nutrients intake probably also facilitate MeHg accumulation in the microbial biomass. Singled-celled organisms represent the major components of marine community, especially in oligotrophic areas such is the Adriatic Sea. In these conditions, small organisms are better competitors for inorganic and organic nutrients because of lower energetic costs, rapid metabolism and small size. Microorganisms might represent a critical Hg bioaccumulation step in the entire marine food web. In order to establish relationship between Hg and microbial species, we performed sampling campaign in the Middle Adriatic Sea. Samplings were conducted from March 2014 to June 2015 during oceanographic cruises aboard the Croatian research vessel Bios Dva. Our research was constrained to transect from the Bay of Kaštela to the island of Vis. We collected non-filtered water samples for determination of total mercury (THg), dissolved gaseous mercury (DGM), methylmercury (MeHg) and picoeukaryotes in Adriatic coastal and open waters. Near the island of Vis, THg concentrations are the lowest and range from 0.14-1.10 ng/L. Mercury contamination from chlor-alkali industrial waters in the Bay of Kaštela is seen through the highest THg concentrations (0.92-5.58 ng/L). DGM is always higher in the contaminated areas (31.8-351 pg/L) than in the pristine environment (22.1-245 pg/L). MeHg concentrations vary, but range from 1.07-34.3 pg/L for all stations. The highest MeHg values are usually found in the Bay of Kaštela. Number of picoeukaryotes is the highest the Bay of Kaštela (0.44×10^6-31.8×10^6/L) which is affected by effluents from the surrounding cities, while the lowest number (0.63×10^6-19.9×10^6/L) is found in the pristine environment of the island of Vis.

TH030

China's current mercury policies and their implications for the overall success of Minamata Convention

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China is currently the biggest emitter of atmospheric mercury, accounting for 1/3 to 2/5 of global emission depending on the study methods. Asia especially China is the net exporter of mercury through atmospheric transport. The projections of atmospheric emission of mercury also shows China's significant role in global mercury emission in 2050. What policies China currently has to reduce the impacts of mercury contamination, and whether there are major gaps in the current policies are key questions for the overall success of Minamata Convention. Here, we reviewed the current Chinese policies related to mercury under the context of Minamata Convention. Based on the information, we assessed compliance situation for the different convention obligations, and identified important policy gaps. China has been aware of the development of international efforts on a legal binding treaty to reduce global mercury pollution and been actively participating in all rounds of intergovernmental negotiations. Currently China has already had a set of policies to reduce mercury contamination and consumption which address most of articles of Minamata Convention. With the increasing awareness, China has a strong policy emphasis on source control, including reducing mercury emission (Article 8), mercury-added products (Article 4) and mercury catalyst used in vinyl chloride monomer industry (Article 5). However fewer efforts have been made for the trade and supply (Article 3), interim storage (Article 10) and waste handling (Article 11), the content of these articles are too a large extent addressed by the current general policy and regulations for all types of waste and chemical management. Though China has already a comprehensive set of policies and regulations which may serve as basis for the implementation of Minamata Convention, there are some important issues which are not addressed sufficiently by the existing policies, including the capacity problem for emission measurement and monitoring, phase-off of mercury containing devices, no retrospective mercury (chemical) management system and lack of information on mercury release to water and soil.

TH031

Use of the RISK21 Matrix: Impact of Mercury Species Exposure to Fish and Seafood on Human Health Risk in Taiwan

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Methylmercury (MeHg) is a highly toxic mercury species and associated \nwith variety of adverse health effects in humans and animals. Fishery resource \nis an important industry in Taiwan. Moreover, fish and seafood are the main \ndietary sources that provide protein, nutrients and omega 3 fatty acids. However, \nexposure to MeHg via fish/seafood consumption is a potential human health \nrisk factor worldwide. Therefore, it is important to be able to prioritize fish and \nseafood products on decisions making of fishery management for government \nand to quantify the MeHg exposure to fish and seafood for health risk \nassessment. \nThe purposes of this study were threefolds: (i) to estimate the intake of \nMeHg and total mercury (THg) via fishs and seafoods in all age groups, (ii) to \nprioritize which fishs and seafoods should be considered for human health risk, \nand (iii) to assess the culinary treatments of fish and seafood for assessing the \nhealth risk of MeHg and THg. \nThe exposure data were collected to assess different age groups intake \nTHg/MeHg concentrations from published papers, research papers, and the \nnational food consumption database in Taiwan. The toxicity information of \nMeHg and THg was based on toxicity data of contaminants from United States \nEnvironmental Protection Agency Integrated Risk Information System on-line \ndatabase. The RISK21 risk visualization matrix was used to assess estimates \nof exposure and toxicity together that can provide a clear view of human safety \nand risk. Finally, a probabilistic risk assessment framework was used to predict \nMeHg intake risk on culinary treatments of fish and seafood. \nThe preliminary results indicated that (i) consumption of saltwater fish has \nmore safety concern than that of freshwater fish and (ii) children and elderly \ngroups should be further evaluation than adolescent and adult groups by using \nthe estimates of toxicity based on available data. We suggest that the RISK21 \nmatrix serves as a useful tool to communicate with stakeholders and \ngovernment. KEYWORDS: Methylmercury, Fish, Risk Assessment, RISK21 Matrix

TH032

Dissolved organic matter controls mercury photoreactions in freshwater lakes

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Methylmercury (MeHg) contamination through bioaccumulation and biomagnification is an issue in many remote ecosystems far from direct pollution sources. Quantifying why and how some ecosystems are more sensitive to contamination following atmospheric mercury deposition is key to mercury fate modeling. Once bacterially methylated and transported into the water column of lakes photodemethylation of MeHg is thought to be one of the main processes in which MeHg can be converted into a less biologically toxic form. While previous studies highlight the importance of photodemethylation to mercury budgets, few have examined the magnitude and variability of photodemethylation rates as a function of associated dissolved organic matter (DOM). DOM absorbs specific wavelengths of solar radiation and therefore MeHg that is bound to these compounds containing photoreactive functional groups may be more readily degraded than unbound MeHg. Alternatively, DOM may absorb much of solar radiation in the top few centimeters of the water column and inhibit photodemethylation with depth. A temporal comparison study between summer and fall was conducted using lake water collected from 6 lakes in Kejimkujik National Park, Nova Scotia, Canada. Sample lakes were chosen based on a known range of DOM concentration. Lake waters were filtered to 0.45 µm and placed in closed polytetrafluoroethylene (PTFE) bottles with >50% headspace, spiked with 3 ng/L MeHgOH, and exposed to 0, 1, 2, 3, 5, and 7 days of natural solar radiation in each experimental season. Solar radiation and water temperature were measured every 5 minutes for the duration of the experiment. Ultraviolet (UV)-visible absorbance scans (200-800 nm), DOM concentrations, total mercury concentrations, and MeHg concentrations were measured for each time point. Dark controls had no significant reduction in MeHg concentration (all p's>0.1) supporting the hypothesis that all MeHg losses in the clear bottles were due to photodemethylation. Photodemethylation rates between lakes were compared

using ANCOVAs and showed that lakes with higher DOM concentrations had significantly lower rates of photodemethylation than lakes with lower DOM concentration ($p < 0.001$). Climate change in temperate and boreal regions of Atlantic Canada is projected to increase rainfall amounts and occurrences and thus lead to browning of freshwaters and further inhibition to the photodemethylation pathway of MeHg reduction.

TH033

Mercury concentration in PM10 collected in the Metropolitan Area of Mexico City

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The anthropogenic emissions are leading to a general increase of mercury in the environmnet. Urban areas are particularly affected since they are exposed to wide diversity of sources, Chemical information obtained from monitoring programs is relevant to identify emission sources. In order to evaluate the contribution of Hg to environmental pollution of the Metropolitan Area of Mexico City, airborne particles ≤ 10 µm (PM_{10}) were collected at four different sites in the urban. Samples collected during 2004 to 2014 were selected to be analyzed. Mercury determinations were carried out by inductively coupled mass spectrometry (ICP-MS). Average Hg concentration in the analyzed particulate matter are 77.0 pg m^{-3} in the. No spatial trend is evident.

TH034

Divalent metal resistance of mercury resistant bacterial isolates and their potential for mercury bioremediation

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Three mercury resistant bacterial isolates were tested for the presence of mercury resistant genes in their chromosomal DNA, and evaluated for their toxic response to divalent metal ions specifically cadmium (Cd), lead (Pb), zinc (Zn) and copper (Cu) in liquid media. The genes involved in mercury resistance namely *merT*, *merP*, *merR* and *CYC* were detected, amplified and sequenced from the bacterial isolates. The deduced amino acid sequences of mercury resistant genes demonstrated homology with genes from a diverse microbial group. Isolate *Sphingobium* SA2 and *Sphingopyxis* SE2 showed resistance to all tested divalent metals to varying degrees while grown in individual metal supplemented low phosphate media. Whereas isolate *Pseudoxanthomonas* SE1 did not show tolerance to the divalent metals. For isolate *Sphingobium* SA2, the 72 h EC_{50} values of Cd, Pb, Zn and Cu were 13, 26, 62 and 3 mg/L respectively; whereas for isolate *Sphingopyxis* SE2 these values were 3, 27, 3 and 5 respectively. Since mercury contaminated soil and water are likely to contain other divalent heavy metals, the multi-metal resistance of mercury resistant bacterial isolates SA2 and SE2 show great potential for their application as bio-agents for the remediation of mercury contaminated soil and water.

TH035

Mercury bioavailability and toxicity to green microalga

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Bioavailability is a key concept allowing to link the changes in contaminant concentrations and speciation with the biological responses. It is also central for the understanding of Hg incorporation in the aquatic food web and possible implications for the environmental health. Nonetheless, in the specific case for Hg exposure the capabilities of the bioavailability to green microalgae, representing the base of the aquatic food chain in aquatic systems is not fully understood. The present study, therefore, explores the links between the chemical speciation, bioavailability and short-term induced effects of inorganic Hg (IHg) and methyl Hg (MeHg) to microalga *Chlamydomonas reinhardtii* under environmentally relevant exposure concentrations. The bioavailability was characterized by intracellular Hg contents at 10^{-10}, 10^{-9} and 10^{-7} M IHg or 5×10^{-11}, 5×10^{-10}, 5×10^{-9} and 5×10^{-8} M MeHg. It was further linked to the following biological endpoints: (i) the oxidative stress (ii) the photosynthesis efficiency and the differential gene expression. Chemical speciation in the exposure medium was also calculated and linked to the bioavailability. Obtained results demonstrated an increase of the intracellular Hg and MeHg contents with the concentration in the exposure medium. The number of up or down regulated transcripts increased with increasing intracellular Hg contents of both IHg and MeHg. At the similar intracellular content, the number of the affected transcripts was higher for MeHg than IHg at similar bioaccumulation supporting that MeHg has a stronger impact on algae than IHg. Furthermore, exposure to MeHg increased photosynthesis efficiency and up-regulated of many genes involved in this biological pathway for all studied concentrations, while IHg induced similar effects but only at highest exposure concentration. MeHg induced increase of the percentage of the cells experiencing oxidative stress, while no oxidative stress was detected for IHg exposure. Overall the present results demonstrated the stronger response of algae upon MeHg exposure as compared to IHg for comparable intracellular concentrations. The outcomes of this basic research contribute to the development of early warning biomarkers in support to biomonitoring efforts of mercury monitoring programs in aquatic systems.

TH036

Dietary transfer of Hg from Elodea nuttallii to Gammarus fossarum

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In the aquatic environment, the organic form of Hg, methyl-Hg (MMHg) and inorganic Hg (IHg) are both toxic, but MMHg has been shown to biomagnify through food chain while IHg does not. Nonetheless, the precise factors controlling the transfer of Hg from primary producers to heterotroph consumers remains poorly understood. Macrophytes are key organism of shallow aquatic environment in which MMHg is produced, and have been identified as having a role in Hg transfer to food webs. The objective of this study was therefore to assess how the compartmentation of IHg and MMHg in a representative macrophyte, *Elodea nuttallii*, affects their bioaccumulation through dietary transfer in *Gammarus fossarum* chosen as surrogate species for aquatic herbivores and decomposers. Bioaccumulation of Hg in *E. nuttallii* was higher for cell wall than intracellular in line with previous studies showing an important role of binding of Hg in cell wall in *E. nuttallii*. Our data suggest no substantial demethylation or methylation during bioaccumulation in *E. nuttallii in planta* or in water. THg and MMHg concentrations increased in Gammarids in correlation with concentration found in *E. nuttallii*. MMHg was more transferable than IHg, based on THg, but a significant part was demethylated during the feeding, while no methylation was observed in the IHg exposure. Cell wall results in higher concentrations than intracellular, suggesting that Hg in cell wall in form of MMHg and IHg was significantly assimilated by Gammarids. Significant uptake fluxes were estimated by the first-order model: uptake fluxes in Gammarids were higher for cell wall than intracellular, while similar uptake rates were observed for IHg and MMHg. In conclusion, *G. fossarum* is able to feed on *E. nuttallii* and accumulates Hg from intracellular and cell wall compartments. In line with literature, when looking at THg, consumption of MMHg-exposed plants leads to slightly higher accumulation of Hg than IHg-exposed plants, but we observed a significant demethylation of MMHg in Gammarids *vs* plants. Opposite to literature, we did not observed a significant biomagnification of MMHg. In sum, although the subcellular metal distribution is determinant for many consumers that are unable to assimilate the insoluble fraction of cell walls, the digestive strategies to handle food of the consumer is also important. Differences in primary producers and consumers species composition may significantly influence the fate of Hg in food webs.

TH037

Bird colonies as secondary sources of Hg in Antarctic ecosystems: the use of vegetation and soil as indicators

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"Global distillation" is classically pointed as the biggest responsible by contaminants input in Polar ecosystems. Besides that, mercury (Hg) presents natural sources as well, in a lesser scale. Nevertheless, the concentration of Hg along the life of organisms (bioaccumulation), and also along the trophic webs (biomagnification), combined with the fact that individuals gather in large numbers and excrete on land are particularly noticeable for seabirds during the reproductive season. Only a few studies proposed such colonies as an important secondary contaminants source for terrestrial or aquatic Polar ecosystems. In a previous study using vegetation as indicator we showed that seabird colonies constitute non-negligible sources of organic contaminants. In this context, the present work evaluates the influence of seabird colonies as secondary sources of Hg using both soil and vegetation as indicators in maritime Antarctic ecosystems, considering geographical variation, distance to the colonies and the use of different bird species with different feeding ecology strategies. Samples of lichen (n=55), mosses (n=58) and soils (n=37), were collected in 13 locations within the South Shetlands Archipelago in the austral summers of 2013-14 and 2014-15 and divided in two types: "colony" (within the colony itself for soil and bordering it for vegetation) and "control" (at least 50m away from the respectively closest colony). Preliminary results (dry weight) for lichens range from 0.077 to 0.705 $\mu g.g^{-1}$, with control samples generally less contaminated than colony ones. There were however exceptions i.e., the locations close to research stations, probably due to fossil fuel burning, and the locations in Deception Island, subject to volcanic activity. Mosses, in turn, range from 0.018 to 0.389 $\mu g.g^{-1}$. Contrary to lichens, they are more water-dependent and are not only subject to dry deposition, but also to contaminants in the water-phase and normally occur in less exposed and often ice-covered areas (except during the summer), remaining less exposed to evaporated/resuspended/lixiviated Hg from colonies. The concentration distribution was roughly the same as for lichens. For soils, results range from 0.005 to 0.302 $\mu g.g^{-1}$, with no exceptions: control samples were always less contaminated than the colony ones. These patterns may indicate colonies as possible Hg sources. Further data treatment will allow to estimate and quantify the influence of those variables.

TH038

Influence of Avian Biovectors on Mercury Speciation in a Wetland

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Mercury is a persistent and bioaccumulative chemical that is present in many remote environments due to its ability to be transported long distances in the atmosphere, and to be deposited far from the original source (Sunderland and Chmura 2000). Wetland ecosystems are important "hot spots" for mercury in eastern Canada, providing anoxic environmental conditions that promote the bacterial methylation of mercury. Methyl mercury is the most biologically available form of mercury and the form which biomagnifies in food webs (Gochfeld 2003). Seabird guano is a well-documented biovector for metals – including mercury – and nutrients, which may indirectly affect metal speciation (Choy et al. 2010). The site for this study, Big Meadow Bog (Brier Island, Nova Scotia, Canada) has a history of ditching in the 1950s, which changed hydrology significantly, resulting in colonization by 3000 pairs of herring gulls (*Larus argentatus*) in the 1980s. To quantify changes in mercury mobilization and speciation in response to this biovector, groundwater samples were collected from this site as well as a reference bog with similar geological and hydrological characteristics. The filtered samples were analyzed for total mercury, methyl mercury, and water chemistry (pH, conductivity, anions, cations, and dissolved organic and inorganic carbon). Preliminary results show significantly higher nutrients (nitrate, phosphate, and sulfate), total mercury, and methyl mercury concentration when compared to the reference bog that is minimally impacted by avian biovectors. This elevated availability of methyl mercury could potentially pose a threat to the local ecosystem and wildlife population due to methyl mercury's toxicity to living organisms (Akearok et al. 2010, Singh et al. 2011).

TH039

Toxicity Reference Values for Methylmercury Effects on Birds: Critical Review and Analysis

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Effects of mercury on birds have been studied extensively and with increasing frequency in recent years. Some highly exposed populations have exhibited adverse effects related to environmental methylmercury exposures while other species are relatively tolerant. We conducted a systematic review of methylmercury effects on bird reproduction, evaluating laboratory and field studies in which observed effects could be attributed primarily to mercury. Our review focused on exposures via diet and maternal transfer in which observed effects (or lack thereof) could be associated with mercury concentrations in diet, eggs, or adult blood. While dose-response data sets were limited to a few species, no observed effect levels, lowest observed effect levels, and/or effect thresholds (e.g., effects of approximately 20%) could be identified for more than 20 species. From this data set, we identified ranges of toxicity reference values (TRVs) suitable for risk assessment purposes. For mercury in avian blood, this represents the first broad compilation of relevant toxicity data. For dietary exposures, the current data support TRVs that are higher than older, commonly used TRVs. The older diet-based TRVs incorporated conservative assumptions and uncertainty factors that are no longer justifiable, although they were appropriate due to limited data at the time those TRVs were derived. The egg TRVs identified from this review are more similar to other previously derived TRVs but have been updated to incorporate new information from recent studies.

TH040

Mercury trend governed by fish population demography?

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Mercury (Hg) is a toxic element entering the biosphere from natural and anthropogenic sources. Mercury in Atlantic cod (*Gadus morhua*) is one of many things that are monitored through the Norwegian contribution to the Coordinated Environmental Monitoring Programme (CEMP) carried out by the Norwegian Institute for Water Research (NIVA) by contract from the Norwegian Environment Agency. CEMP is administered by the Oslo and Paris Commissions (OSPAR) in their effort to assess and remedy anthropogenic impact on the marine environment of the North East Atlantic. Time series for cod in the Inner Oslofjord (Norway) goes back to 1984. Annual median Hg-concentrations in cod from the Inner Oslofjord (Norway) showed both significant upward long-term (whole time series) and short-term (last 10 years) trends. However, the median length of the cod sampled also showed an upward trend. This is consistent with results of beach seine surveys (dating back to 1919) conducted in the Inner Oslofjord, showing that cod recruitment in the area has been low since the start of the 2000s. To investigate how length would impact the trend analysis, the Hg-concentrations in the cod fillet were normalized to two arbitrary lengths: 40 cm and 60 cm. No significant trend in Hg-concentrations could be detected for either 40 cm-normalized, or 60 cm-normalized cod the last 10 years. The results indicated that most of the upward trend in Hg-concentrations could be attributed to the catching of larger fish. The influence of other explanatory variables than length needs to be explored. It is not merely aquestion of why mercury is apparently increasing in cod, but also what are the reasons for the apparent change in the cod population demography. In this regard, sampling bias must also be considered.

TH041

The fate of mercury legacy in the contemporary environment; From old mines to brown trouts

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Due to their geological features mountain environments have often been exploited since the beginning of metallurgy and the French Pyrenees is no exception. Results on trace metal concentrations in the Ariège region, where extensive mining (Ag, Fe) occurred from the Antiquity to the 19th century, indicates that ≥600 tons of anthropogenic Pb is stored in organic soils on the Pyrenean northern slopes. Similar conclusions can be drawn for other mining legacy pollutants (Hg, Zn). The fate of these trace elements in relation to climate or abrupt environmental changes (e.g. flood, forestry) is poorly understood. Once released from surrounding organic soils the catchment can be highly enriched in the bioavailable fraction of these metals causing a bioaccumulation of e.g. MeHg in river biota. In combination with predicted rise in global atmospheric mercury deposition, the mercury legacy from old mines may pose a severe threat to the biota in these already sensitive high altitude areas. E.g. preliminary results show that levels of tot-Hg in brown trout (*Salmo trutta*) caught at our study sites surpasses litterature values by 10 times or more. Further to this, MIF and MDF Hg-isotope signatures shows clear relationship with the size of the fish and also with $\delta^{15}N$. By linking data from atmosphere – soil – peat – water – sediment – biota, and the transfer between these continuums, we are studying the fate of legacy pollutants (Pb, Ag, Bi, Sb, Hg, As and U) with a main focus of Hg, in three mountain catchments (Etangs de Bassies, Largentière and Bernadouze) all located in the French Pyrenees. Our study also includes biofilm, invertebrates, common minnow and brow trout representing various aspect of the biotic food chain. We aim to infer the potential risk these legacy pollutants pose on river biota (i.e. fish) and environment at large. To do so we combine traditional geochemical analysis (DMA, HR-ICP-MS) with stable isotopes (Pb and Hg, $\delta^{13}C$ & $\delta^{15}N$) and analysis of radiocarbon and radionuclides (^{210}Pb, ^{137}Cs, ^{241}Am and ^{7}Be) in order to investigate the origin of these elements. We also perform micronucleus analysis of the fish blood to test if any toxicological effects can already be seen. Our goal is to assess the potential harmful exposure these legacy pollutants in general and Hg in particular, may pose to the environment, to humans and to society at large.

TH042

Specific pathways and effects of dietary methylmercury and inorganic mercury in zebrafish (Danio rerio) determined by mercury speciation, genetic, histological and metallothionein responses.

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A multidisciplinary approach is proposed here to compare toxicity mechanisms of methylmercury (MeHg) and inorganic mercury (iHg) in muscle, liver and brain from zebrafish (*Danio rerio*). Animals were dietary exposed to (1) 50 ng Hg. g^{-1}, 80% as MeHg; (2) diet enriched in MeHg 10000 ng Hg. g^{-1}, 95% as MeHg; (3) diet enriched in iHg 10000 ng Hg. g^{-1}, 99% as iHg, for two months. Hg species specific bioaccumulation pathways were highlighted, with a preferential bioaccumulation of MeHg in brain and iHg in liver. In the same way, differences in genetic pattern were observed for both Hg species, (an early genetic response (7 days) for both species in the three organs and a late genetic response (62 days) for iHg) and revealed a dissimilar metabolization of both Hg species. Among the 18 studied genes involved in key metabolic pathways of the cell, major genetic responses were observed in muscle. Electron microscopy revealed damage mainly due to MeHg in muscle and also in liver tissue. In brain, high MeHg and iHg concentrations induced metallothionein production. Finally, the importance of the fish origin in ecotoxicological studies, here the 7th descent of a zebrafish line, is discussed.

TH043

Determination of mercury and methylmercury concentrations at low levels in

rivers by passive sampling method and isotopic dilution GC-ICP-MS

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Diffusive gradient in thin film (DGT) samplers have been widely used as water quality monitoring tools to study cationic trace metals of the Water Framework Directive (WFD) (lead, cadmium, nickel). However, these samplers based on a polyacrylamide diffusive gel and a Chelex resin do not allow trapping methylmercury (MeHg) and inorganic mercury [Hg(II)] which is also a priority substance of the WFD. The most common DGTs developed for these two mercury species are based on a 3-mercaptopropyl functionalized silica gel (3M) resin, with a diffusive gel either in polyacrylamide or in agarose. However, previous studies focused on the application of such DGTs in relatively contaminated surface waters (total Hg in dissolved phase HgT_D >5 ng/L or MeHg in dissolved phase $MeHgT_D$ >0.25 ng/L). The aim of this study was to develop a home-made passive sampler combined with analysis by gas chromatography-inductively coupled plasma-mass spectrometry (GC-ICP-MS) to measure low levels of mercury species in surface waters. Consequently, our work first focused on the control of Hg contamination mainly via the control of blank DGT. Then, we developed an analytical method coupling isotopic dilution with GC-ICP-MS, which allowed the extraction, derivatization and quantification of the mass of Hg(II) and MeHg trapped by the home-made/3M DGT at environmental levels. For in-situ validation, triplicates of home-made samplers were deployed at three sites in a low contaminated river (HgT_D~0.5 ng/L), and we assessed time-weighted average concentrations (TWAC) for labile HgT_D and $MeHgT_D$. Our home-made/3M DGT resins contained significantly lower level of total Hg (Hg_{TOT}) (< LQ=0.03 ng) compared to commercial DGTs based on a spheron-thiol resin. These results clearly demonstrated that the initial mass of Hg in commercially available DGTs was masking Hg accumulation after deployment in a low contaminated river. Thus, in these environmental conditions, commercial DGTs are not well adapted. In contrast, our home-made/3M DGTs were suitable to assess TWAC of HgT_D in a low contaminated river. Then, the quantification of Hg(II) and MeHg trapped in the home-made/3M resin was achieved by isotopic dilution (ID), extraction with acidic thiourea solution and derivatization before measurement by GC-ICP-MS. This optimized, sensitive and reproducible method, combining passive sampling method (10 days deployment) and ID-GC-ICP-MS, allowed the measurement of low level of labile HgT_D and $MeHg_D$ (90-130 pg/L).

Mechanistic toxicology of engineered nanomaterials: state of the art and future perspectives (P)

TH044

Natural organic matter influences UV light induced toxicity of nano-sized titanium dioxide

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The constantly growing application of nano-sized titanium dioxide ($nTiO_2$) leads to an increasing entry in aquatic ecosystems, e.g. through waste water effluents. Effects caused by $nTiO_2$ on aquatic life, however, vary substantially in their intensity, which is often attributed to particle characteristics. A well-known property of $nTiO_2$ is the formation of reactive oxygen species (ROS), inducing toxic effects at ambient UV light irradiations and low $nTiO_2$ concentrations. At the same time, natural organic matter (NOM) is suppressing the formation of ROS. Yet, only limited knowledge is available on the interaction of $nTiO_2$ with UV light and NOM. Therefore, this study assessed the toxicity of $nTiO_2$ (P25; ~90 nm; 0.00-128.00 mg/L) when interacting with increasing levels of UV light (0.00-5.20 W UVA/m²) and NOM (seaweed extract; 0.00-4.00 mg TOC/L) using acute toxicity tests with *Daphnia magna* as a model system. Results supported the hypothesis of a decreased effect of $nTiO_2$ in presence of NOM, whereas the presence of UV light increased the toxicity with an increasing intensity up to 280-fold (i.e., at 4.80-5.20 W UVA/m²) when compared to experiments in absence of UV. This pattern was attributed to amplifying ROS formation under higher UV light intensities. However, the $nTiO_2$ induced ROS formation was not inversely proportional to increasing NOM levels, i.e. lower concentrations (0.04-0.40 mg TOC/L) diminished, whereas a higher concentration (4.00 mg TOC/L) promoted the ROS quantity, irrespective of UV light intensity, which partly contradicts the results obtained by the *Daphnia* bioassays. Therefore, this study demonstrated that the combination of UV light and NOM had the potential to modify the direction and intensity of the effects caused by $nTiO_2$ relative to a consideration of impacts of UV light and NOM individually. Since the trigger for this joint effect of UV light and NOM on the toxicity of $nTiO_2$ remains unclear, further research on nanoparticle toxicity should be conducted with UV light and varying quantities and quality of NOM to uncover the governing mechanism influencing the formation of ROS.

TH045

Assessing aqueous phase silver nanomaterial (Ag NM) environmental fate; a flume based approach

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Flumes offer the ability to study Ag NM removal and persistence behaviour in controlled and potentially 'environmentally relevant' scenarios. Studying removal rates within flow systems may produce fate descriptors to translate processes into useful models which will help with exposure assessment in realistic conditions. The aim of this research was to develop flume based systems and methods to investigate aqueous phase Ag NM fate and behaviour within different aquatic conditions, and subsequently investigate the effects of flow, organic and inorganic matter, the presence of non-cohesive sediments and re-suspensions events on aqueous phase Ag NM removal/persistence and re-dispersion. Two Ag NM were studied within flume systems (flow velocity 0.12 m/s): Ag NM (PVP) (30-50nm), and analogous reference material NM300 (15±5nm), within two recirculating turbulent flow test systems containing 1.8 L and 55 L of soft water medium, respectively. Studies were carried out over a time course of up to 24 days. At the end of the experiment flow velocity was increased, when relevant, to entrain the bed sediment and create re-suspension events. Suwannee river humic acid (SRHA), Sigma humic acid and kaolinite clay were used as model organic and inorganic matter, and silica sand was also used as a model non cohesive sediment. Total aqueous phase Ag concentration (to assess removal), primary particle size of NM, aggregation state and zeta potential, and dissolution were investigated at specific time points in soft water medium, using a range of suitable methods. Results indicated NM300 removal rates and aggregation state within the aqueous system increased within turbulent flow relative to static systems, indicating turbulent flow significantly increased removal processes, which was attributed to increased sedimentation rates. The addition of kaolinite clay (20 mg/l) and humic acid (2 mg/l) did not significantly increase or stabilise Ag NM aqueous phase removal within flow conditions. However higher concentrations of humic acid (10 mg/l) resulted in longer term persistence of NM300 within the water column. The presence of non- cohesive sediments significantly increased removal rates of NM300. Ag NMs exhibited re-dispersion following re-suspension events. These results indicate that turbulent flow would be the dominating factor in modulating Ag NM removal in lotic water, when organic matter concentrations are low, and remobilisation of Ag NM needs futher consideration.

TH046

Influence of soil pH and plant specie in the leaching of Zn in two crops developed in soils spiked with ZnO-NPs, ZnO-bulk and ZnSO4 under greenhouse conditions.

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Nanotechnology is becoming increasingly important in the agricultural sector. Promising results and applications of nanoparticles (NPs) are already being developed in the areas of delivery of pesticides, fertilizers and genetic material for plant transformation. However, their use is not without risk (toxicity to ecosystems, phytotoxicity to crops and effects on both soil and water quality) which should be carefully evaluated. In recent years, rapid progress has been achieved in the study of phytotoxicity of NPs and accumulation in plants, but important issues still remain to be solved. Among them, the importance of the physicochemical soil characteristics for NPs toxicity and their potential to contaminate water compartments due to the transfer of contaminants from soil to water. The aims of this study were to evaluate the influence of soil pH and plant specie in the leaching of Zn, in two crops developed under greenhouse conditions, and in two soils spiked with ZnO-NPs, ZnO-bulk and $ZnSO_4$. A lysimeter experiment was carried out in two agricultural soils: an acidic soil (pH 5.4) and a calcareous soil (pH 8.3), using two plant species (*Solanum lycopersicum* L. and *Phaseolus vulgaris* L.) under greenhouse conditions. The upper part of the soil (3 kg of a total of 10 kg) were spiked with 3, 20 and 225 mg Zn kg^{-1} as ZnO-NP; 20 and 225 mg Zn kg^{-1} as ZnO-bulk; and 20, 100 and 225 mg Zn kg^{-1} as $ZnSO_4$. The soils were irrigated at slightly above field capacity moisture to obtain 6 portions (each of 400 mL) of leachate. The average Zn concentrations in the leachate were much higher in the acidic soil than in the calcareous soil. However, there were no differences with respect to the crop. According to LSD test ($P < 0.05$), in the acidic soil, total quantities of Zn in the leachate depended on the Zn treatment applied. There were no significant differences among total quantities of Zn leached from ZnO-bulk and ZnO-NPs treatments, for the same dose. However, these amounts were significantly higher for soils spiked with $ZnSO_4$ (except for the dose of 20 mg Zn kg^{-1}). The amount of leached Zn in treatments with doses less than 20 mg Zn kg^{-1} was comparable with that leached from the control. Regarding the amounts of total Zn leached for the same period in the calcareous soil, there were no significant differences among the treatment or crops with respect to total quantities of Zn in the leachate. This research was funded by the Spanish projects RTA2013-00091-C02-02 and RTA2013-00091-C02-01

TH047

Ecotoxicology of Sediment-associated Carbon Nanotubes.

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Carbon nanotubes (CNTs), single-walled (SWCNTs) and multi-walled (MWCNTs), are high aspect ratio nanostructures with a combination of properties making them useful in an increasing number of products and applications. Although CNTs occur in the environment as the result of natural combustion processes, significant environmental exposure to engineered CNTs would previously not have occurred and therefore they are considered xenobiotics. Investigations of CNT toxicity are complicated by factors such as the large variety and synthesis methods, as well as the lack of standardized testing procedures, but also their tendency to agglomerate in aquatic systems. Few *in vivo* studies have examined the behaviour of CNTs in marine systems and their bioavailability and toxicity to marine organisms. We have previously shown that SWCNTs show relatively low toxicity to marine mussels; the main concern was that SWCNTs influence the toxicity of other contaminants at otherwise benign concentrations. CNTs in the aquatic environment are expected to rapidly precipitate and become incorporated into sediments. The aim of the present study was to assess the behaviour of CNTs in sediments, their bioavailability to benthic species and their effect on the bioavailability and toxicity of other sediment-associated contaminants. Experiments showed that CNTs ($500\mu gL^{-1}$) could be recovered and confirmed using Raman spectroscopy (RS), albeit with very weak spectra; we are currently improving the technique. Ecotoxicological effects of sediment-associated SCNTs and MCNTs (dispersed in 0.02% SRNOM) were investigated by injecting them into seawater tanks (pH7.9-8; 20°C) containing light-coloured play pen sand washed playpen sand. CNTs were left to settle on to the sediment surface, after which cockles (*Creastoderma edule*) were introduced and left to bury and filter-feed the nephloid layer for 72hrs. Uptake of CNTs was confirmed using RS and TEM imaging. A suite of biomarkers of exposure were studied, including oxidative stress (SOD, CAT, TBARS, GSH, GPx) and associated genotoxicity. CNTs settled down very quickly after one hour and no changes in their behaviour were observed after 24 hr. Early genotoxicity data suggest that sediment-associated CNTs caused an increase in DNA strand breaks in *C. edule* at concentrations $>100\mu gL^{-1}$. Work is currently ongoing to extend the exposure concentration range down to more environmentally relevant levels and to test the causative oxidative stress hypothesis.

TH048

Investigating the effects of nanomaterials from waste water treatment plants; removal, release and subsequent impacts

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The majority of nanomaterials (NMs) used for commercial applications will enter the waste water treatment plant. In many countries the waste water effluent and sewage sludge are discharged in aquatic environments or applied in agricultural land, however, the transformation of the particles and the potential hazard they pose in these compartments are poorly understood. The main aim of the study is to understand the transformation of NMs during the waste water treatment processes and to evaluate the potential environmental hazard of the transformed `aged´ particles compared to the pristine ones. Initial investigations focus on the study of silver and titanium particles incorporating the use of iosotopically labelled particles as well as unlabelled, to better understand the behaviour and fate of these particles in complex media and biological systems. An integrative approach is taken using organisms of multiple trophic levels representing the aquatic and terrestrial environment. A biodynamic study is envisioned for the assessment of uptake, elimination, bioaccumulation and intracellular localization of the NMs both in the pristine form as well after their transformation during the waste water treatment processes. Moreover, *in vitro* cell culture models of high relevance for nanoparticle toxicity assessment representing the main sites of nanoparticle uptake and excretion such as the gills and liver have been developed and incorporated in order to identify possible modes of action.

TH049

Impact of silver and silver nanoparticles on the structure and functionality of microbial communities in sewage treatment plants

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Silver nanoparticles (AgNPs) are amongst the most commonly used nanoparticles in consumer products. Therefore they will inevitable end up in the environment mostly through the entry pathways of sewage sludge to soils (application to fields as a fertilizer) or effluent to freshwater systems. However, due to the antimicrobial properties of silver, the microbial communities in the sewage treatment plants might be affected resulting in a decrease of functional efficiency of the treatment processes. Standard tests to evaluate potential effects on microbial (sludge) communities (e.g. OECD 209) only considers short term effects with average contact times of only 3 hours. Silver is non-biodegradable and therefore has the potential to accumulate in the environment resulting in potential long-term effects on microbial communities. Aim of this study was to compare the effects of two concentrations (1 mg/L & 50 mg/L) of ionic silver ($AgNO_3$) and AgNPs on the community structure over a time period of 2 weeks. The selected silver nanoparticles were commercially available (Amepox, Poland), uncoated and paraffin stabilized with a primary particle size of 3-8 nm. The aqueous AgNP stock suspension (1000 mg/L) was characterized with DLS, NTA, TEM (size distribution and homogeneity) and AAS (for dissolved and total silver concentration). The diversity of the bacterial community and potential structural changes were analyzed with 16srRNA sequencing (dual index sequencing/MiSeq). Activated sludge was incubated for 2 weeks (aerated; fed every 3 days with meat extract) and samples for sequencing were taken at 4 time points (start (0), 2, 7 and 14 days) to track structural community changes. Silver concentrations were monitored in parallel for dissolved (post ultracentrifugation) and total silver concentrations by atomic adsorption spectroscopy (AAS).Analytical results showed in general a low total and dissolved silver concentration in the water phase staying relatively constant over the testing period suggesting that most of the silver was rapidly bound to the organic matrix at the beginning of the test. Microbial communities showed structural differences between the ionic and the silver nanoparticle treatments, but only after 7 days. The effects were most distinct for the 50 mg/L AgNO3 treatment (after 7 days). For all other treatments (1 mg/L $AgNO_3$/1& 50 mg/L AgNPs) significant effects were visible only after 14 days supporting the importance to analyse long-term effects.

TH050

The influence of soil properties on long-term effects of silver and silver nanoparticles on soil microbial communities

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Metal and metal oxide nanoparticles (NPs) are currently the nano materials with the highest total production volume. Silver nanoparticles (AgNPs) have gained considerable attention due to their broad microbiocidal properties, which implies a specific hazard for exposed soil bacteria. Both chemical transformations of the NPs (e.g. dissolution) and the chemistry of the soil may modify the organism exposure and response in NP–dosed soils. Previous work has shown that soil type influences toxicity of ZnO NPs to microbial communities with soil pH playing a major role. In this study we investigated how soil properties influence the bioavailability and toxicity of silver (Ag) NPs and ionic silver to natural soil microbial communities. Soils were dosed with either 50nm diameter PVP-coated Ag NP or an ionic silver reference, silver nitrate ($AgNO_3$). Four soils were used for this study with a soil organic matter (SOM) content ranging from 1.8–16.7% and soil pore water pH from 4.5–8.3. Microbial communities were exposed to nominal soil concentrations ranging from 9 – 5500 mg Ag/kg. The diversity of the bacterial communities and potential structural changes were analyzed with 16s rRNA sequencing (dual index sequencing/MiSeq) at 4 time points; at test start, after 4 weeks, 8 weeks and 52 weeks. Soil pore waters were extracted from all soils at the beginning (t=0days) and after 8 weeks of the experiment, ultra-filtered (10kDa) and analysed for silver, pH and dissolved organic carbon. Results clearly showed an influence of silver and silver nanoparticles on soil microbial communities and clear differences in the community structure between the ionic and the AgNPs treated soils. At the beginning of the test effects of $AgNO_3$ were more distinct than for AgNPs, but with increasing exposure time these differences became less distinct, suggesting gradual dissolution of the AgNPs and a corresponding increasing impact of the released ions on the microbial community structure. Soil pH was identified as one of the key properties influencing bioavailability and toxicity of silver and AgNPs on the microbial communities. Our results also show that long-term effects (e.g. slow NP dissolution in soils over months) need to be considered for a realistic environmental hazard assessment of AgNPs.

TH051

Coating relevance in the nanoparticle toxic effect to Daphnia magna.

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Coating of nanoparticles (NPs) with a proper surface agent is usually required before their use in any application, to improve its functionality. This coating will determine the NP interaction with the environment as the physicochemical properties of the NPs can be modified due to this cover, leading to different effects in organisms. A major regulatory problem will be the high amount of assays that

should be conducted for all coating-NP combinations. In the framework of FP7 Project GUIDEnano we investigated the toxicity produced by different coatings on three metal NPs (synthetized from PlasmaChem GmbH, Germany) to try to establish relationships between coatings and toxicity. The acute effect of NPs, in terms of core composition (TiO_2, CeO_2 and Ag), was investigated on neonates of *Daphnia magna* after 48 h exposure following the OECD guidelines. The two first NPs were functionalized with three different coatings: citrate (TiO_2-CIT/CeO_2-CIT), polyethylene glycol (TiO_2-PEG/CeO_2-PEG), and dodecylphosphonic acid (TiO_2-DDPA/CeO_2-DDPA), moreover those NPs were tested also uncoated (TiO_2-UNC/CeO_2-UNC). AgNPs were coated with CIT, PEG and oleylamine (HPB). Both DDPA and HPB confer hydrophobic characteristics to the metal NPs, whereas CIT and PEG make NPs more hydrophilic. The highest immobilizing effect was found after exposure to AgNPs, showing the lowest IC_{50}. In those cases, assuming that Ag-CIT can behave as the uncoated NP, the toxic effect of the AgNPs increased by their functionalization with PEG and HPB. On the other hand, *D. magna* exposed to TiO_2-UNC and CeO_2-UNC presented higher toxicity than the same core coated with CIT, PEG or DDPA where no toxic effect was observed. Thus the coating conducted to a protective effect over the metallic core. The size of the NPs in the exposure medium could not explain this different behavior between metal-oxide and metal NPs. It is more likely that the coating interaction with the core lead to a different behavior of the NPs. From this study a specific pattern linked to the coating could be established depending on the core composition. The coatings of the metal-oxide nanoparticles eliminated completely the toxicity produced by the uncoated NPs. On the contrary, toxicity of the metal NPs was increased depending on the coating surrounding the AgNP.
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TH052
The effects of a panel of nanomaterials on the freshwater microalga Pseudokirchneriella subcapitata
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The increased use of nanomaterials in a wide range of products is likely to lead to larger releases of nanomaterials into the environment. The aim of this study is to assess the effects of a panel of nanomaterials on the freshwater microalga *Pseudokirchneriella subcapitata* in order to evaluate the aquatic toxicity of nanomaterials. Recent studies have compared the toxicity of nanomaterials of varied physico-chemical properties to algae and investigated the impact of the dispersion protocol on nanomaterial toxicity. Results indicate that the physico-chemical properties of nanomaterials (e.g. composition, size, solubility) are able to influence their toxicity and that exposure and effects depend on media and the approach used to suspend the nanomaterials. In this study *P. subcapitata* was exposed to widely used nanomaterials (silver, single wall carbon nanotubes, multi wall carbon nanotubes and nanocelluloses) at different concentrations (20 - 200 μg/L, for silver nanomaterials and 0.1 to 100 mg/L, for single wall carbon nanotubes, multi wall carbon nanotubes and nanocelluloses), established following pilot studies. A silver salt ($AgNO_3$) control (3 to 17 μg/L) was also tested for comparison purposes with silver nanomaterials. Nanomaterials were dispersed using bath or probe sonication, following well established and published protocols. The growth of *P. subcapitata* was measured following OECD test guideline 201, at different time points (0, 24h, 48h, and 72h), via measurement of optical density (OD) and fluorescence, as well as chlorophyll a, after extraction. Median effective concentrations (EC_{50}) at the different time points revealed that silver nanomaterials were more toxic in terms of growth inhibition than carbon-based NMs, although silver salt was found to be more toxic than the silver nanomaterials. The dispersion method used was found to be a key factor in determining toxicity, with the probe sonicator protocol resulting in higher toxicity. Results from this work indicate the importance in establishing clear standard methods when assessing the hazard of nanomaterials to the aquatic environment.
Key words: nanomaterials, toxicity, sonication and *P. subcapitata*.

TH053
Comparative effect of ZnO-NPs, ZnO bulk and ZnSO4 in the antioxidative defence system of two plant species growing in two agricultural soils in a greenhouse experiment
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The nanoparticle (NP) application in food production and agricultural industry is emerging. The use of NPs in agriculture aims mainly to reduce applied amounts of plant protection products, minimize nutrient losses in fertilization and increase yields trough an optimized nutrient management. Therefore, a lot of attention is currently paid to the potential risks arising from the use of these materials in agriculture. In the last years numerous studies have evaluated the phytotoxicity of NPs. However, most of these studies have been performed in hydroponic media over short time periods. These studies do not mimic field conditions and may overestimate toxicity. Phytotoxicity studies under more relevant exposure conditions are necessary due to the importance of the medium and the environmental conditions on the availability and, hence, the toxicity of NPs. Moreover, the studies covering the whole cycle of a planta are desirable to determine the long-term effects. One the most discussed mechanisms by which NPs have toxic effects on organisms is the generation of reactive oxygen species (ROS) which result in oxidative stress to organism. Several studies have shown evidence of oxidative stress on plants exposed to soils contaminated with NPs, but more information is necessary, especially in realistic exposure conditions. The effects of ZnO-NPs on oxidative stress of two plants species of agronomic interest: tomato (*Solanum lycopersicon*) and bean (*Phaseolus vulgaris*) were studied and compared with ZnO bulk and $ZnSO_4$ in a greenhouse experiment. The effects were assessed in two agricultural soils with different pH (an acid soil pH 5.4 and a basic soil pH 8.3) to evaluate the influence of the physicochemical soil properties in the NP toxicity. The soils were spiked with 20 and 225 mg Zn kg^{-1} soil. Additionally, 3 and 100mg Zn kg^{-1} soil were tested for ZnO-NPs and $ZnSO_4$, respectively. Changes on the levels of ROS and proteins, the activity of the antioxidant enzymes (APX, GPOD and CAT), and the effects on lipid peroxidation were measured at different exposure times (15, 30, 60 and 90 days). The results showed that ZnO-NPs could cause oxidative stress in plants under greenhouse conditions. These effects depended on the plant species, soil pH and the exposure time. However, differences between ZnO-NPs, ZnO bulk and $ZnSO_4$ were not observed with some exceptions. This research was funded by the Spanish project RTA2013-00091-C02-01 and RTA2013-00091-C02-02.

TH054
The toxicity of differently coated copper oxide nanoparticles towards the marine blue mussel, Mytilus edulis.
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Rapid growth in the field of nanotechnology is continually increasing the potential release of nanoparticles (NPs) into the environment. Although copper oxide nanoparticles (CuO NPs) are widely used within numerous industrial and commercial applications (e.g. batteries, electronic circuits, superconductors, solar energy conversion and gas sensors), their toxicity is still poorly understood in comparison with other metal oxide NPs. As the main recipients of industrial and domestic wastewaters, aquatic systems will inevitably become a sink for CuO NPs throughout their life cycle. Filter-feeders, such as the blue mussel, (*Mytilus edulis*)are widely used in environmental monitoring and are organisms of great interest in regards to the fate and toxicity of NPs within the aquatic environment. This study, funded by the FP7 project, NANOSOLUTIONS, aims to investigate the mode of toxicity exhibited by CuO NPs towards *M. edulis* and the effect of surface coatings upon CuO NP toxicity. *M. edulis* were exposed to core CuO NPs and CuO NPs coated with polyethylene glycol, nitrate and carboxylic acid, for 48 hours at concentrations of 10 and 20 μgL^{-1}. Following exposure, the activity of the antioxidant enzyme superoxide dismutase (SOD) was assessed in *M. edulis* gill and digestive gland tissues to determine whether reactive oxygen species (ROS) were formed as a result of CuO NP exposure, whilst the TBARS (Thiobarbituric Acid Reactive Substances) assay was used to determine whether lipid peroxidation (a product of oxidative stress) had occurred. Additionally, ASCGE (alkaline single cell gel electrophoresis) comet assays were performed on gill and haemolymph cells to assess potential DNA damage caused by CuO NP exposure. It was hypothesised that an increase in CuO NP concentration would lead to an increase in *M. edulis* SOD activity, lipid peroxidation and DNA damage, whilst coatings would influence CuO NP toxicity. Work is currently underway to test these hypotheses.

TH055
Influence of size and surface coating on silver nanoparticles uptake by Gammarus fossarum
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The increasing production and use of silver nanoparticles (AgNPs) will inevitably lead to their release in aquatic environments where they represent a threat to aquatic species. Therefore, there is still a need to better understand the mechanisms underlying the potential toxicity of AgNPs. Being a very important component of the macroinvertebrate assemblage, *Gammarus fossarum* will certainly be exposed to AgNPs if they reach the water streams. Therefore, *G. fossarum* was selected as a model organism in order to assess AgNPs effects. This study evaluated the acute effects (72h) of three different sizes of AgNPs (20, 40 and 80 nm), either stabilized with citrate (CIT-AgNPs) or coated with polyethylene glycol (PEG-AgNPs), on adult male *G. fossarum*. Tested AgNPs concentrations ranged from 0.5 to 50 ug.L^{-1}. $AgNO_3$ was used to compare AgNPs effects with silver ion effects. $AgNO_3$ was the most toxic compound for *G. fossarum* with an LC50-72h of 1.68 ug.L^{-1}, while none of the studied AgNPs led to lethal effects. Size distribution of CIT-AgNPs and PEG-AgNPs in exposure

media was characterized using nanoparticle tracking analysis. CIT-AgNPs 40 nm and CIT-AgNPs 80 nm were stable whereas CIT-AgNPs 20 nm aggregated to NPs of 53 nm. In contrast, all PEG-AgNPs were stable. Uptake of silver by *G. fossarum*, assessed by ICP-MS, revealed a surface-coating dependent effect with CIT-AgNPs being taken up to a greater extent than PEG-AgNPs. Additionally, a size dependent uptake was observed with CIT-AgNPs 40 nm being more taken up than CIT-AgNPs 20 nm and CIT-AgNPs 80 nm. The same tendency, linking the size to the uptake, was observed for PEG-AgNPs with higher uptake of PEG-AgNPs 20 nm than PEG-AgNPs 40 nm and PEG-AgNPs 80 nm. These results show effects at low and environmentally realistic concentrations of AgNPs for *G. fossarum* and confirm the hypothesis that silver uptake is dependent on size and surface coating of AgNPs.

TH056

The disposition of cationic amino polystyrene nanoparticles in sea urchin immune system cells and their effect on multixenobiotic resistance phenotype

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The increasing discard of micro and nanopolymers in the oceans can seriously impact marine ecosystems. Our previous results showed that cationic amino polystyrene nanoparticles (PS-NH_2) induce both embryotoxicity in the sea urchin *Paracentrotus lividus* and immunomodulation in mussel hemocytes. The sea urchin immune system cells (coelomocytes) have been emerged as biosensor in ecotoxicology studies. However, there is no data regarding the effect of polystyrene nanoparticles on these cells. So, the aim of the present study was to investigate the disposition of PS-NH_2 in the sea urchin *P. lividus* coelomocytes and their effect on multixenobiotic resistance phenotype (MXR). The behavior of PS-NH_2 in coelomic fluid medium was assessed by DLS analysis. Coelomic fluid was withdrawn with a 1 mL syringe pre-loaded with anticoagulant solution (500 µL ISO-EDTA). Cells were then centrifuged, resuspended in coelomic fluid, and cell concentration adjusted to 1 x 10^6 cells/mL. Coelomocytes were exposed to PS-NH_2 (1 to 25 µg/mL) and nanoparticle disposition analyzed under fluorescence microscopy. MXR phenotype was investigated by the calcein intracellular accumulation assay. PS-NH_2 was shown to accumulate just in phagocytes and in a time dependent manner. Vibralite cells, red and colorless sphere cells did not exhibit nanoparticle accumulation or plasma membrane adsorption. No cytotoxicity was observed to PS-NH_2 up to 10 µg/mL and until 24 h of cell exposure. However, the PS-NH_2 highest concentration induced cell death (loss of membrane integrity). Moreover, PS-NH_2 exposure - up to 24 h - did not increase calcein intracellular accumulation in coelomocytes. Our data suggest that PS-NH_2 uptake is an active and selective process since only phagocytes internalized the nanoparticles. However, no cytotoxic effect was observed when coelomocytes were exposed to environmentally relevant concentrations. Furthermore, PS-NH_2 exposure, even in the highest concentration, did not affect MXR phenotype. The preliminary results show that exposure to PS-NH_2 up to 10 µg/mL do not impair *P. lividus* immune system cells viability.

TH057

Fate and toxicokinetics of differently coated silver nanoparticles in the deposit-feeding polychaete Capitella teleta

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Aquatic sediments are believed to constitute an important sink to silver nanoparticles (AgNPs). Knowledge on the effects of AgNPs to sediment-dwelling organisms together with their behavior and fate in these environments are therefore necessary. In this study, the effects and toxicokinetics of differently coated AgNPs (un-coated, polyvinylpyrrolidone-coated (PVP) and mercaptoundecanoic acid-coated (MUDA)) added to sediment in nominal concentrations of 100 μgAg/g dry weight sediment were tested on the sediment-dwelling polychaete *Capitella teleta*. After 15 days of exposure, survival and growth of *C. teleta* was not affected by any of the AgNPs and uptake of the AgNPs was generally low with average weight specific body burdens (WSBB) of 0.140 ugAg/g dry weight worm tissue. After 15 days of depuration in clean sediment, WSBBs had been reduced with more than 50% in all treatments. There was a tendency that MUDA-coated AgNPs were most available for uptake and un-coated AgNPs were least. The fate of the AgNPs in the test system was assessed by measuring the distribution of Ag in the different experimental compartments after 7 and 15 days. The results showed that Ag was mainly recovered in sediment and fecal pellets. Silver concentrations were 2.6 to 3.7 times higher in fecal pellets than in sediment. Fecal pellet Ag concentration was lowest for MUDA-coated AgNPs and highest for un-coated AgNPs. The results suggest that fate and uptake of AgNPs are influenced by particle coating and that *C. teleta* influences the fate of sediment-associated AgNPs.

TH058

Subcellular partitioning of size-dependent copper nanoparticle in grass carp (Ctenopharyngodon idella)

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A smaller size of nanoparticles (NPs) may show unique nanoscale properties. To date, the size-dependent waterborne copper nanoparticle (CuNP) toxicity and subcellular partitioning information in the edible freshwater fish are poorly studied. To gain insight into how assumptions regarding size-dependent metal NPs influence their ecotoxicities, this study assessed the mode of action in subcellular detoxification of grass carp (*Ctenopharyngodon idella*) exposed to size-dependent CuNPs. This studycarried out the CuNP subcellular distribution experiments with grass carp from natural populations varying with concentrations and three nanoscales of 25, 40-60, and 60-80 nm. Results showed that smaller size CuNPs had higher accumulation and acute toxicity to freshwater fish. The metabolically detoxified pool (MDP) contained the relatively higher percentages of CuNPs over 37.09 – 72.53%, 37.67 – 66.57%, and 44.21 – 62.26% than those of metabolically active pool (MAP) in 25, 40-60, and 60-80 nm treatment, respectively. We found that metal-rich granules contributed most CuNPs accumulation after CuNPs exposure, indicating that it is a key role on CuNPs detoxification. To our knowledge, it is the first study on size-dependent CuNP subcellular partitioning in freshwater fish. We anticipate that the finding of this study could be used to identify a suitable enzyme or protein that has a strong protection on CuNPs susceptibility and to provide the most potentially promising biomarker to detect CuNPs in the aquatic environments.

TH059

In vitro testing for identification of long term effects of copper oxide nanoparticles in fish cell lines

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Copper oxide nanoparticles (CuONPs) have been widely applied in a number of areas and their antimicrobial activity has opened the door to their use as biocides. As a consequence, there is a high likelihood of release of these NPs into waste streams, and further into aquatic systems. Although CuONPs have been described as toxic for aquatic organisms in a number of studies, these experiments were normally carried out for short periods of time, observing effects on one single generation. However, there is a general lack of knowledge about long term toxic effects of NPs, including CuONPs. In this sense, *in vitro* systems can be a powerful tool to initially determine the mechanisms underlying toxic effects of NPs after prolonged exposure. The objective of this work was to explore the cytotoxic effects of CuONPs on fish cell lines after long-term, low-dose exposures. Two fish cell lines were used in this work: a cell line derived from rainbow trout liver biliary ducts (RTL-W1) and CLC cells, derived from carp macrophages. Cytotoxicity was assessed by means of the alamarBlue, neutral red and CFDA-AM assays. First, cells were exposed to a range of CuONPs (prepared and supplied by PlasmaChem GmbH, Berlin) concentrations for 24 h in order to determine the most appropriate concentrations for longer exposure experiments. Those concentrations used in the 24h exposure most close to the EC50 and EC10 were used in the longer term experiments: 100 and 50 μg/ml, respectively for CLC cells, and 100 and 25 μg/ml, respectively for RTL-W1 cells. Exposure lasted for 21 days and cells were split every 7 days, receiving fresh medium with appropriate concentrations of CuONPs. CLC cells exhibited a very high susceptibility towards CuONPs reaching 100% mortality after 7 days exposure to both concentrations. RTL-W1 viability was not affected after exposure to 25 μg/ml CuONPs for 21 days, but 100 μg/ml caused approximately 90% mortality. TEM analysis of exposed cells evidenced a general disruption of cellular organelles and membranes. The *in vitro* protocol used appears to be useful to study the cytotoxic effects of continuous low-dose exposure to different nanomaterials in a more realistic scenario for prediction of their toxicity in higher tier tests, supporting the 3Rs initiative in the development of alternative ecotoxicological tests. Acknowledgements - This research was supported by FP7-SUN project (Grant Agreement No. 604305).

TH060

Ecotoxicity of double-walled carbon nanotubes on Chironomus and Xenopus larvae: monospecific and mesocosm tests

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Carbon-based nanoparticles (CNPs) can be found in many manufactured products and are thus likely to be released in the environment. Among them, double-walled carbon nanotubes (DWCNTs) are well characterized and may be considered as a control nanomaterial to investigate the effects of CNPs on organisms, particularly in the aquatic environment, which is a major receptacle of pollutants.\nThe goal of the present work is (i) to improve the understanding of toxicity mechanisms of a range of concentrations (from 0.1 to 50 mg.L-1 of DWCNTs) on benthic and pelagic organisms by a monospecific approach and (ii) to assess the ecotoxicological risks by using more realistic conditions in a mesocosm, with an interaction between species. DWCNTs are known to interact with constituents of the environment such as natural organic matter (NOM). For this reason, all our tests were performed in the presence of added commercial Suwannee River NOM. Two standardized single-species tests were carried out, respectively, for two aquatic models: *Chironomus riparius* and *Xenopus laevis*. The effect of DWCNTs on monospecific phototroph biofilm of *Nitzschia palea* was tested too. In a second step, these organisms have been grouped within a food chain and exposed to 1 $mg.L^{-1}$ of exposure water. Studied endpoints for chironomids were acute (mortality), chronic toxicity (growth and development delay) and teratogenicity. Furthermore, mortality, growth inhibition and genotoxicity (induction of micronucleated erythrocytes) were studied on *Xenopus*. In monospecific tests, an algal growth delay was measured after 48 h of exposure from $1mg.L^{-1}$. The survival rate of chironomids was significantly impacted from 1 $mg.L^{-1}$. No significant teratogenicity neither growth inhibition was observed. However the frequency of development delay was lower than the negative control from 1 mg L^{-1}. The results didn't show mortality for *Xenopus* but significant growth inhibition and genotoxicity (induction of micronucleated erythrocytes) were noted at 10 $mg.L^{-1}$. Macro-observations suggested that the chronic toxicity could be limited to physical effects (intestinal obstruction and/or abrasive effects and/or nutrients deprivation and/or gill clogging). No effect was observed for all species in mesocosm configuration except a significant growth delay in the chironomid negative control, maybe due to a competition with *Xenopus* for food. Key Words: double-walled carbon nanotubes, ecotoxicity, *Xenopus laevis*, *Chironomus riparius*

TH061

In vitro cytotoxicity of graphene oxide nanosheets on earthworm coelomocytes

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By increasing concerns of carbon based nanomaterials that release to terrestrial ecosystem, toxicity of carbon based nanomaterials on earthworms have been steadily investigaed. However, very limited researches on toxicity of graphene oxide nanosheets on earthworm species were reported. This study investigated *in vitro* cytotoxicity of graphene oxide nanosheets on earthworm coelomocytes. Coelmocytes of *Eisenia andrei* were exposed to three different sized graphene oxide nanosheets via *in vitro* and then intracellular esterase activity, oxidative stress, cell size and cell granularity changes were measured by flow cytometer. The results showed that intracellular esterase activities were inhibited and reactive oxygen species were generated. Also cell size was decreased and cell granularity was slightly increased. It indicates that graphene oxide nanosheets caused *in vitro* cytotoxicity on earthworm coelomocytes. *Acknowledgements. This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (2013R1A1A2061386), and the Ministry of Science, ICT and Future Planning (2014R1A2A1A11050513).This research has also been performed as a cooperation project for 2015 Environmental Risk Assessment of Manufactured Nanomaterials funded by the Korea Institute of Toxicology (KIT, Korea).*

TH062

Rational design of HMPAA towards environmental safety

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The production of manufactured nanomaterials (MNM) has been growing since the 90's to fulfill pharmaceuticals, cosmetics, textiles, informatics component, and many others industry and society needs. The present work intended to evaluate the influence of changes in the structural conformation of hydrophobically modified polyacrylic acid (HMPAA) MNMs in their toxicity to freshwater biota in order to identify the MNM variation that is most environmental friendly. Six variations of HMPAA MNMs were obtained by modifying their crosslinked conformation and the insertion position and length of hydrophobic groups. Their ecotoxicity was characterised using the cladoceran *Daphnia magna* as a model organism. Standardised toxicity assays were conducted in order to assess median effect concentrations for: i) survival, ii) feeding behaviour, iii) juvenile growth rate and iv) intrinsic growth rate. Tested concentrations of each HMPAA variation ranged from 0 to 2000 mgL^{-1} (corresponding to the concentration commercially available). Results showed that, at lethal levels, the variation HMPAA2 (with small and large hydrophobic groups positioned alternately along a linear structure) was the most toxic (LC50, 48h= 203.6 mgL^{-1}); while HMPAA5 (sphere structure with hydrophobic groups inside a dense matrix) did not cause any lethal effects after 48h, at 2000 mgL^{-1}. On the contrary, at sub-lethal levels, HMPAA2 was one of the least toxic variations. HMPAA4 (sphere structure hydrophobic groups disposed at the surface) was the most toxic variation for all the sub-lethal endpoints evaluated: feeding ($EC_{50,24h}$= 111.2 $mgL^{-1)}$, somatic growth ($EC_{50,72h}$= 92,4 mgL^{-1}) and intrinsic growth rate (fewest number of neonates per brood, in the first brood and, therefore, lowest number of total neonates and populational growth – *r*). HMPAA 2 and HMPAA 6 (sphere structure with hydrophobic groups inside a tight matrix) reveal to be the less toxic when analyzing the effects in feeding ($EC_{50,24h}$= 249.2 and 281.2 mgL^{-1}, respectively), though, being more toxic to daphnids growth ($EC_{50,72h}$= 276. 8 and 491.2 mgL^{-1}, respectively).The obtained results suggest that different HMPAA structures influences differently the toxicity of these MNM when present at lethal or sub-lethal levels, which challenges the identification of the variation that is more environmentally safe.

TH063

Report on TiO2 nanoparticles ecotoxicity studies in the framework of REACH regulation

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TiO_2 is one of chemical substances registered under REACH regulation. In the specific framework of substance evaluation, a procedure under REACH regulation, we aim to assess risk and safety of producing and using TiO_2. In this work, an inventory of all studies published between 2010 and 2015 related to TiO_2 ecotoxicity studies was carried out. We chose to focus on the 3 main types of aquatic organisms used in regulation: algae, daphnia and fish. They are used in OECD Test Guideline (TG) under REACH and CLP regulation. Thus, 105 studies have been selected in this work: 15 studies with algae, 60 with invertebrates and 30 with fishes. Several parameters have been chosen to select only reliable studies: well-characterized nanoparticles, condition of exposure according to OECD recommendation with the use of replicates, localization/bioaccumulation of nanoparticles. Among all studies selected, most of the Lethal Concentration 50 (LC_{50}), Effective Concentration 50 (EC_{50}) or No Observed Effect Concentration (NOEC) are higher than 1 mg/L. Nevertheless, eight studies on daphnia revealed LC_{50} or EC_{50} below 1 mg/L of TiO_2 anatase, or P25 (70:30; anatase: rutile). Daphnia appears as the most sensitive organism. The parameters involved in the toxic response are: size and crystalline phase of nanoparticles, complexity of medium of exposure, use of UV or light during exposure, increased exposure time compared to the OECD TG. This work highlights the lack of data on characterization of TiO_2 nanoparticles in published ecotoxicity studies and the fact that a fitting of the OECD TG is needed for the nanoparticles testing case.

TH064

Investigation of the potential uptake of magnetofluorescent nanoparticles into human cells

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The creation of magnetofluorescent nanoparticles (MNPs) for the selective labelling of cancer cells represents a field of interest of computational nanotoxicology. The library including 109 MNPs generated by Weissleder and colleagues in 2005, and the related uptake into human cells (i.e. endothelial cells, activated human macrophages and pancreatic cancer cells), was object of several computational studies aiming to predict the uptake behaviour of these NPs from the structure of the organic compounds used as coating. However, due to the structure of the experimental data, all these studies were mainly focussed on the prediction of the uptake into pancreatic adenocarcinoma cells, and almost no effort was provided to try to exploit the experimental information by using, for instance, multivariate analysis, nor to provide additional insight through the identification and analysis of outlier compounds. In this study multivariate analysis (e.g. Principal Component Analysis) was applied to investigate the macro properties of the experimental dataset originally generated by Weissleder and colleagues in order to study the uptake behaviour of the 109 MNPs into different cell lines. Additionally, models based on Quantitative Structure Activity Relationships (QSAR) were developed to predict the uptake behaviour of these NPs into the human pancreatic adenocarcinoma cells and human umbilical vein cells. These models were built on the basis of traditional structural descriptors calculated from the structures of the organic molecules used as coating. These molecules represent the element of structural variation across the 109 MNPs, which all share the same iron nano-core. QSAR models were generated using several linear and non-linear techniques. The statistical performances of these QSARs were investigated, compared, and used to identify common outliers. The models were validated internally and externally on multiple validation and prediction sets. This was performed in order to investigate the internal robustness of the models, the sensitivity of different methods to perturbations of the datasets, as well as to evaluate the predictive ability of the models on MNPs not included in the models development. This study shows how multiple statistical approaches can be applied to investigate complex datasets, and how different QSAR models can be combined to improve the results of models taken singularly.

Polar ecotoxicology: hot issues in cold climates! (P)

TH065
Distribution and origin of selected PCB and PAHs in the Arctic fjords sediments
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The main objective of the study is to present preliminary results of selected Persistent Organic Pollutants (POPs) concentrations in the Arctic fjords sediments. The extention of knowledge on POPs cycling in the Arctic is important since POPs may be transported over long distances from distant sources, are persistent in the environment and toxic. They tend to accumulate in fatty tissues of organisms, moreover may biomagnify along the arctic food web. POPs are highly reactive particles and are readily sorbed onto sinking organic and mineral particles. Part of them is accumulated by marine organisms and other part is deposited at the sea bottom. Deposited contaminants may be re-introduced to the water column and be again bioavailable for organisms. The study presents the results of polychlorinated biphenyls (PCBs) and polyaromatic aromatic hydrocarbons (PAHs) concentration in sediments collected from Kongsfjord, Hornsund and Adventfjord (eastern Svalbard). These fjords are influenced by different water masses, different rate of glaciers ablation and the intensity of primary and secondary production. In addition, the knowledge on POPs concentrations in the Adventfjord near Longyearbyen may allow to assess the significance of local pollution source. The concentrations of selected polychlorinated biphenyles (CB28, CB52, CB101, dlCB118, CB138, CB153, CB180) and selected polycyclic aromatic hydrocarbons (NAP, FLN, PHE, ANT, FLT, PYR, BAA, CHR, BKF, BAP, DBA, BP, IND) have been measured in sediment cores from selected depth intervals. GC-FID and GC-ECD techniques were used for qualitative and quantitative analysis of polychlorinated biphenyls and polycyclic aromatic hydrocarbons. To assess the origin of PAHs contaminants in sediments, individual components ratios were used. Sediment cores were dated using ^{210}Pb method, therefore the history of POPs accumulation has also been studied. The concentration of Σ7PCBs and ∑12 PAHs in sediments ranged from 0.05 to 1.5 ng/g d.w. and from 33.5 to 463.3 ng/g d.w. respectively. The compounds present in highest proportion were volatile CB28 congener and phenanthrene. The obtained results are also discussed in the context of environmental conditions that may influence POPs accumulation.

TH066
Organochlorine pesticides in archived antarctic ice cores
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Persistent Organic Pollutants (POPs) are toxic ubiquitous anthropogenic substances that have been widely used in agricultural and manufacturing industries since the 1930s. A variety of organochlorine pesticides (OCPs) have been listed as POPs since implementation of the Stockholm Convention which bans or severely restricts the use of POPs into the environment to minimise the hazard they pose. POPs are highly resistant to biodegradation, toxic and bioaccumulative. They are also capable of long-range environmental transport which has led to primarily atmospheric transport of part of the global POP burden to remote Polar Regions. At high latitudes, cold conditions combined with long periods of darkness diminish their volatility and promote their persistence serving to restrict further movement. The polar cryosphere is thus frequently referred to as a “polar sink” for POPs. In the current global warming context, Polar climate may be significantly altered possibly leading to re-volatilisation of historically deposited POPs. Under this scenario, global efforts to moderate human and environmental exposure to these toxic compounds would be altered and Polar Regions would thus act as secondary sources to the atmosphere. Very limited research has been undertaken to gauge the current load of the POP ice reservoir in Polar Regions, particularly in Antarctica. While the release of POPs from melting glaciers has been observed in alpine areas around the world and recent findings also show the presence of POPs in Arctic ice and OCPs in Antarctic glacier melt-water, this study is the first to determine Antarctic continental ice loading of OCPs. Continental ice cores were collected at Law Dome, East Antarctica. They were transferred to a clean stainless steel melting-unit sealed under high purity nitrogen. Melt-water was filtered and subsequently analysed for a suite of 31 OCP analytes. Two early deposition periods were studied 1945-1957 and 1958-1967. Our methods allowed detection of OCPs in both periods studied, particularly for α- and δ-HCH, heptachlor, chlorpyrifos, trans-chlordane, dieldrin, endrin and endosulfan in the dissolved fraction of the meltwater. We observe differences between the two time period studied, sensibly corresponding to usage in Australia where development and use of OCPs began in the mid-1940s, and emissions peaked between 1960 and the mid-1970s. Results and implications will be further presented and discussed.

TH067
Using environmental metabolomics to assess the contribution of oil droplets to dispersion toxicity in pelagic zooplankton Calanus finmarchicus (Crustacea: Copepoda)
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Toxicity of oil to marine organisms is driven by the presence of dissolved oil components and their partitioning to organisms. In oil dispersions and produced water, a large fraction of the oil components are particle-bound and their potential contribution to toxicity is neglected. For filter-feeding organisms, like copepods, particulate oil may be ingested and as such represent an additional route of exposure. In an attempt to separate the toxic effects of dissolved and dispersed fractions on copepods, we treated them with three concentrations of oil dispersions (D) and filtered oil dispersions to provide a water-soluble fraction (W) without droplets. We also included controls which were fed algae like the exposed copepods, and controls that were not fed (starved). After exposure, copepods were sampled for NMR metabolomics analyses. Initially, Principal Component Analysis (PCA) was performed on the NMR data. The first two principal components (cumulative 69.49%) were dominated by variations in the dataset not associated with the treatments (PC1, 44.45%) and variation which clustered the treatments but not according to dose (PC2, 25.04%). PC3, at 10.67% of the variation separated both high dose dispersion and dissolved treatments from control. In addition the starved group clustered together with these groups. The Kruskall-Wallis rank sum test gave $p< 0.05$ and the following Games-Howell post hoc test gave significant differences for the medium dose dispersion (MD) ($p=0.05$). The high dose dispersion (HD) and high dose water solubles (HW) were also different from the control with means in the same range as MD but with larger variances possibly causing $p>0.05$. The starved group was not significant at the $p=0.05$ level ($P=0.059$) but with a similar mean value as for the MD, HD and HW groups. The PC3 loading profile showed shifts in amino acids which to a large extent had loading values to indicate lower occurrence in high dose exposed and starved groups. Osmolytes taurine and betaine showed the same, whereas homarine had a loading value to indicate higher occurrence in exposed copepods. From these results, exposure to MD, HD and HW fractions was found to give similar global metabolic responses as with starvation as observed by the PCA approach used here. The fact that only the highest dose W group was different from the control whereas both MD and HD were different could indicate that the dispersions are more potent in inducing responses in the metabolome.

TH068
Effects of produced water components on Arctic copepods
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Calanoid copepods such as *Calanus finmarchicus*, *C. glacialis* and *C. hyperboreus* are the dominating zooplankton species in the North Atlantic Ocean and the Barents Sea. They store excess energy by accumulating large volumes of lipids during their late development stages before molting into adults. This discrete lipid sac is utilized during periods of low food availability and for reproduction. Exposure to lipophilic water pollutants such as polyaromatic hydrocarbons (PAHs) can lead to the sequestration of these compounds inside the lipid sac. Very low elimination rates of PAHs have been observed in *C. finmarchicus* after exposure to produced water (PW) components warranting further studies on potentially delayed implications of these compounds on survival during diapause and reproductive success. As oil exploration and production moves further north towards the Arctic, the risk of such effects increases and an understanding of the toxicokinetics of PW components on Arctic keystone species is crucial for risk assessment. Key findings of our previous work investigating the effects of petrogenic components on *C. finmarchicus* were revised to provide reference data for our latest project, PWC-Arctic. The main aim is to increase the knowledge on the potential effects of dispersed oil and other PW components on growth, development and reproduction in the Arctic copepods *C. glacialis* and *C. hyperboreus*, thereby supplying parameterized toxicity data for risk assessment of petroleum activity in theArctic. This will be achieved by describing the potential for accumulation (bioavailability) of petroleum related contaminants and dispersed oil droplets from PW and relate this to potential effects on assumed sensitive early life stages.

TH069
Uptake and elimination kinetics for 11 PAHs in Arctic lipid rich copepods
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Oil exploration and production has recently moved further north towards the Arctic, increasing the potential for exposure of Arctic organisms to polycyclic aromatic hydrocarbons (PAHs) during regular production as constituents of produced water (PW) and from accidental oil spills. Hence, understanding the toxicokinetics of PAHs in Arctic species is increasingly important for environmental risk assessments of regular discharges and damage assessment following accidental spills. The calanoid copepods *Calanus finmarchicus*, *C. glacialis* and *C. hyperboreus* are the dominating zooplankton species in the North Atlantic Ocean and the Barents Sea. They store excess energy by accumulating large volumes of lipids during their late development stages before molting into adults. Previous studies have shown that exposure to lipophilic water pollutants such as PAHs results in an accumulation of these components in *Calanus* species. Traditionally the uptake and depuration kinetics are modelled using one-compartment toxicokinetics. However, previous experimental data on *C. finmarchicus* strongly indicate that elimination of PAHs is much slower than expected based on calculations by state-of-the-art one-compartment toxicokinetic models such as OMEGA. In the present study, the lipid rich developmental stage copepodite V (CV) of the Arctic *Calanus glacialis* was exposed to a filtered dispersion of alkane oil spiked with 11 PAHs for 120 h at 1 °C. The log Kow of the PAHs ranged between 3.3 for naphthalene and 5.78 for chrysene. A 120 h depuration period in clean seawater followed the exposure period, and samples for body burden analyses were taken at regular intervals during both the exposure and the depuration periods. Models developed to fit to the uptake data were used to predict depuration kinetics for individual compounds. Several of the PAHs, especially the heavier fluoranthene, pyrene and chrysene, showed approximately 100 % retention after the 120 h depuration period, substantial deviations from the predictions by the one-compartment models. The slow depuration of PAHs in lipid rich Arctic copepods demonstrated in this experiment indicates that stored PW components may be more available for transfer to progeny, as well as having a higher potential for transfer to higher trophic levels than previously anticipated.

TH070

Enzyme expression in Antarctic krill (Euphausia superba) exposed to p,p'-DDE

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Despite the apparent pristine nature of Antarctica and it's remoteness to industry, Persistent Organic Pollutants (POPs) have been detected in Antarctic biota since the 1960s. Antarctic krill from the Southern Ocean, an important Antarctic prey species, have been found with notable concentrations of POPs, including the pesticide, DDT and its most common metabolite, *p,p'*-DDE. Several organism-level responses have been observed in krill following sublethal *p,p'*-DDE exposure. However, nothing is currently known about the underlying subcellular mechanisms of these responses, or the metabolism of *p,p'*-DDE in Antarctic krill. The purpose of this study was to examine the metabolic response of Antarctic krill exposed *p,p'*-DDE. Krill were examined for Glutathione S-transferase (GST), Glutathione peroxidase (GPx) and Acetylcholinesterase (AChE) activity in response to *p,p'*-DDE exposures ranging from 1-20 μg/L. None of the enzymes responded to *p,p'*-DDE in a concentration dependant manner. GST activity slightly increased for all doses but only the highest exposure induced a significantly increase. GPx followed a similar trend although none of the concentrations induced a significant increase in activity. Krill exposed to these concentrations exhibited signs of toxicity, most likely due to neurological impairment. This indicates that Antarctic krill exhibit a limited capacity for GST facilitated metabolism of *p,p'*-DDE at these concentrations. AChE decreased in three of the four treatments, but actually increased for the 10μg/L exposure, suggesting that the responses were not dependent on *p,p'*-DDE but may have actually been caused by an underlying factor. Thus AChE inhibition is not an indicator of organochlorine induced neurological toxicity in krill. Based on the GST and GPx activity measured in this study, krill appear not to have the ability to detoxify themselves of *p,p'*-DDE. This may indicate that this species is highly sensitive to this pollutant in the environment and possibly similar POPs. As *p,p'*-DDE is commonly detected in Antarctic biota, the apparent lack of detoxifying ability of Antarctic krill is troubling and should be examined further.

TH071

Baseline energy-budget model for the marine copepod Calanus finmarchicus

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Calanoid copepods form an important part of the marine zooplankton, and are exposed to stressors such as pollution resulting from oil and gas exploration. Mechanistic models are needed to interpret the effects of such stressors, and to predict the impact on the copepod life histories under realistic environmental conditions. Dynamic Energy Budget (DEB) models are well-suited for this task as they can provide an integrated framework covering all life-history traits over the entire life cycle of a species. Furthermore, these models are (relatively) simple, in principle not species specific, and easily linked to population models. Copepods, however, have several features in their life cycle which require further consideration in energy-budget models. Firstly, they develop through six naupliar stages, which deviate in morphology from the later six copepodite stages, and the first two stages do not feed. Secondly, copepods follow determinate growth, and stop growing after their final moult to adulthood. And thirdly, many copepods (especially those species from higher latitudes) build up a considerable lipid storage during the last few copepodite stages to survive adverse environmental conditions and to fuel the reproduction process. Before we can consider the effects of stressors on the life history, we need to develop a baseline model to capture these deviations from the standard DEB models. In this contribution, we report on our progress in modelling the growth, development and lipid storage in *Calanus finmarchicus*, a common species in the Northern Atlantic Ocean and expanding up into the Arctic. Once this model is established, it can serve as a platform to interpret the effects of toxicants and other stresses, and as a basis for extrapolating to other high-latitude copepod species.

TH072

Exposure of ovigerous female copepods to oil causes transcriptional responses in offspring - Maternal transfer of PAHs?

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Copepods of the *Calanus* genus have a large potential for accumulating oil components during exposure due to their high lipid content and impressive filtration rates, and they have been shown to filter and ingest oil droplets during exposure. Female copepods produce eggs at the expense of their discrete lipid sac, suggesting either a remobilization of lipid-accumulated oil components and/or a direct transfer route of such contaminants to offspring. In an attempt to assess the potential for maternal transfer of oil components we exposed ovigerous female copepods (*Calanus finmarchicus*) to filtered and unfiltered oil dispersions for 4 days and collected their eggs the 3-6 days following the end of the exposure period. They were reared in clean water through to the late naupliar stages (N5-6) and collected for RNA extraction and preparation of libraries for high-throughput transcriptome sequencing. Differentially expressed genes were identified through pairwise comparisons between treatments. Expression of 173 genes was altered in response to dispersion exposure, 184 in response to the filtered dispersion, and 35 genes were affected by both treatments. Among the differentially expressed genes, several have known roles in responses to chemical stress including Phase I enzymes (oxidizing, Cyp2J-like), Phase 3 enzymes (exporting, multidrug resistance proteins), antioxidants (thioredoxin, xanthine dehydrogenase), chaperones (Hypoxia upregulated protein, quinone oxidoreductase), and components of the inflammatory response (phospholipase A2 activating protein).

TH073

Plastics in the Antarctic environment (PLANET project): preliminary observations of microplastics in the Antarctic biota

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Several studies estimates that trillions of plastics are floating all over the oceans and thus recognized as a global environmental problem and one of the most important threats for marine ecosystems. Although Antarctica has been historically seen as a pristine region, it might be reached by plastic debris due to the increase of fishing and tourism but also by transport from transboundary sources which can cross the Antarctic Polar Front. Macroplastics (> 1 cm) have been surveyed by sight in the Southern Ocean since 1980s and more recently reported around Antarctica (South Georgia), therefore their presence and high persistence might pose a serious concern for this fragile marine ecosystem. Smaller plastic pieces in the micro and nanosize range can be uptaken and retained by Antarctic organisms, leading to bioaccumulation, toxicity but also trophic transfer to top-predators with potential impact for marine ecosystems as a whole. Up to date, no information for Antarctic marine ecosystem on the presence of micro and nanoplastics are available. While microplastics are quite well studied,

fate and impact of nano-sized plastics in the marine environment are almost unknown and are raising concern due to increasing abundance in water column and their properties which could imply toxicity to marine biota. Therefore, studies in remote areas as Antarctica are urgently needed in order to understand their distribution and take measures for the conservation of one of the last pristine areas in the world. The aim of the PLANET project is to study *i*) the presence of micro and nanoplastics in Antartic marine environment and *ii*) the impact on marine species in terms of bioaccumulation, trophic transfer and toxicity. Several biological samples collected in the last 10 years on the Antarctic Ross Sea area close to the Italian Antarctic Base "Mario Zucchelli" and from a penguin colony at Edmonson point were processed for identifying the presence of micro and nanosized polymers. Samples of phytoplankton, krill, scallop, fish, penguin (blood and feaces) and sea bird (skua) were homogenized, filtered and analysed by light microscopy and by FTIR and Raman for polymer types identification. Fibers and foils were the most abundant and appears to be common across a range of different species and biological samples while sphere were present only in selected samples. Sources, trophic transfer and size able to exert toxicity towards Antarctic marine species are reported and discussed.

TH074

DNA damage in arctic avian predators: baseline, sensitivity to stress and association to contaminant exposure

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The polar region functions as a pollution sink where chemicals produced and used in southern latitudes are transported and deposited. Seabirds are exposed to an increasing load of stress due to a warming climate, loss of habitat, changes in their marine food sources and also pollution. Seabirds are important study organisms in ecotoxicological studies and they are considered good indicators of the oceans health as they inhabit different trophic levels, have different physiological adaptions and may be sensitive to human induced environmental changes. While the legacy pollutants are decreasing in the environment, they are still found in higher concentrations compared to newer compounds that are emerging due to increased chemical use to meet demands from our modern society. Organohalogen contaminants are known to elicit a wide range of negative effects on wildlife, such as effects on enzyme-, immune-, endocrine- and vitamin systems. The field of genotoxicity can provide new information about the effect that pollutants have on an organism's DNA. DNA damage can be measured as a result of different stressors on an organism, and may be an interesting biomarker as an organism's genome is vital for a wide range of different functions and systems. The comet assay is a well-established method to quantify single- and double strand breaks in the DNA; it is relatively easy and inexpensive, and a sensitive tool for assessing DNA damage. The comet assay is used in human toxicology on different types of cells, on other mammals, crustaceans, mollusks and fish. However, few studies have been conducted using the comet assay in assessing genotoxicity in avian wildlife and thus, little is known regarding baseline DNA damage in various species, the sensitivity to oxidative stress-induced damage and the relationship between contaminants and degree of DNA damage. These knowledge gaps are addressed in the present study, focusing on the arctic seabirds the common eider, black-legged kittiwake, arctic skua, and glaucous gull. Field work was conducted in June- July 2015 in Kongsfjorden in Ny Ålesund, Svalbard, and the four different species were caught during incubation or early hatching period. Biometric data was measured and blood samples were taken. Data presented at the SETAC meeting will be levels of DNA damage (baseline and sensitivity) in the different species, and the relationship to drivers behind the observed results; contaminant levels, body condition or other factors.

TH075

Determination of mercury speciation and isotopic composition of Antarctic seabird tissues: Tracing Hg trophic sources or metabolic response?

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Mercury (Hg) is considered a globally distributed pollutant of major concern for humans and wildlife especially under its form of monomethylmercury (MMHg), which is capable to accumulate in the organisms and biomagnify within the food webs. In the last decade, the measurement of Hg isotopic mass dependent and independent fractionation (MDF and MIF, respectively) has become an essential tool in identifying sources of Hg and quantifying its reactivity within the different compartments of the environment. Although Antarctic and sub-Antarctic environments have been usually perceived as remote areas untouched by anthropogenic pressure, they are reached by contaminants through ocean circulation and atmospheric transport. However, Hg contamination in this region remains largely unknown, and new studies combining Hg speciation and isotopic approaches are required to elucidate this question. Seabirds, as upper predators, are exposed to large quantities of Hg via food intake and have been identified as effective biomonitors of Hg marine contamination. This work is focused in the evaluation of Hg sources, trophic transfer and metabolic pathways by analyses of different tissues of seabirds from the French Southern and Antarctic Lands (Southern Indian Ocean). Organs (pectoral muscle, liver, brain and kidney), feather and blood samples were studied by the combination of Hg isotopic composition analyses by Multicollector(MC)-ICP-MS and Hg speciation analyses by species-specific Isotope Dilution Gas Chromatography-ICP-MS (ID-GC-ICP-MS). An optimisation and validation of the sample preparation procedure was previously accomplished. Four seabird species with contrast feeding ecology were studied: Antarctic prion (zoo-plankton eaters), white-chinned petrel and king penguin (mesopelagic fish consumers), and northern giant petrel (other-seabird meat and carrion eaters). Significant differences in MDF signatures were observed between tissues for all the species, in particular for Antarctic prions. An enrichment in lighter δ^{202}Hg values was observed from feathers to muscles and livers which could be attributed to inter-organ transport or metabolic processes. Hg MIF values differed widely between organs which could be the result of different MMHg sources. This study evidences the great potential of the combination of Hg speciation and isotopic composition analyses using seabird tissues to investigate Hg sources and metabolic pathways at different levels of the marine food web.

TH076

Contaminant exposure in arctic foxes (Vulpes lagopus) from Svalbard in relation to climate-linked changes in feeding habits and food availability

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The arctic fox is a circumpolar species and the population inhabiting Svalbard is among the highest contaminated apex predators of arctic ecosystems. At Svalbard arctic foxes are top predators and scavengers that depend on both the terrestrial and marine food web. Climate-induced variability is likely to influence arctic fox diet through availability of reindeer carcasses and sea ice cover. In the present study, temporal trends of a wide range of contaminants in arctic fox (*Vulpes lagopus*) from Svalbard, Norway, sampled 1997-2014, were investigated in relation to feeding habits and food availability. Stable isotope of carbon (Î^{13}C) was included as a proxy for marine versus terrestrial feeding habits. Annual number of carcasses of Svalbard reindeers (*Rangifer tarandus platyrhynchus*) and sea ice cover (access to seals) were used as proxies for food availability. Liver concentrations of all compounds increased with increasing intake of marine food. Reindeer mortality was negatively related to concentrations of perfluorocarboxylic acids (PFCAs), HCB and THg, while perfluoroalkyl sulfonates (PFSAs), Ƭ-HCH and THg concentrations increased with sea ice cover. Linear models revealed that concentrations of PFCAs and THg adjusted for Ᵽ^{13}C, reindeer mortality and sea ice cover increased over time, while no trend was observed for hexachlorobenzene (HCB) and beta-hexachlorocyclohexane (Ƭ-HCH). Adjusted concentrations of PFSAs, polychlorinated biphenyls (PCBs) and their hydroxylated metabolites (OH-PCBs), chlordanes, p,pH_-DDE, mirex, PBDEs decreased over time. The present results suggest that climate-related changes in feeding ecology influence contaminant exposure in arctic foxes.

TH077

In vitro and in silico techniques as a tool to assess effects of pollutants on polar bear energy homeostasis

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Prolongation of ice-free summer seasons will lead to longer fasting periods of polar bears, which use sea ice as a platform for hunting. Fasting is physiologically demanding and requires optimal control of energy homeostasis. Peroxisome proliferator-activated receptors (PPARs) play a central role in regulating energy homeostasis. We hypothesize that contaminant exposure in polar bears may affect their energy homeostasis and thus decrease their ability to respond to climate change. To explore our hypothesis, we used multiple parallel approaches including in silico and in vitro techniques to investigate effects of contaminant mixtures on energy homeostasis of polar bears. Binding of over 600 new and legacy contaminants to polar bear PPARs was studied using *in silico* docking and scoring methods. The results indicate that several brominated and fluorinated compounds have a potential to bind polar bear PPARs. Activation of polar bear PPARs by emerging and legacy chlorinated and brominated contaminants has been studied using *in vitro* cell lines. The results suggest that several common pollutants found in polar bears and humans have either weak agonistic or antagonistic potential towards polar bear PPARs. Synthetic mixtures reflecting the concentrations of contaminants found in polar bear adipose tissue suppressed polar bear and human PPARγ activity as well as PPARγ-mediated accumulation of lipids in mouse fat cells. Lipid accumulation in the mouse fat cells, through PPARγ-independent pathways was not affected following exposure to the synthetic mixtures. Contaminant mixtures extracted from polar bear tissues enhanced lipid accumulation in the mouse fat cells. We have also isolated polar bear stem cells from their adipose tissue and established a method to study lipid accumulation and mRNA expressions in cells exposed to mixtures of contaminants. In addition, we have determined the presence of a wide range of new and legacy compounds in polar bear fat samples by using non-target and target analyses.

Soil and water contaminants: evaluation, biomonitoring and bioindicators for effective management (P)

TH078

The validation of analytical methods as an evaluation tool of research results reliability

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Biochem-Env is a platform for analytical biochemistry created in 2012 by INRA with the support of the program "Investissements d'avenir" ANAEE-France. The mission of Biochem-Env is to provide facilities for the biochemical characterization of natural environments (enzyme activities in soils and sediments) and associated macrofauna (enzyme activities, phospholipid fatty acids, terrestrial and benthic macrofauna biomarkers), as well as lipidic biomarkers (PLFAs). Partner in research projects, the platform must produce traceable analytical data with high level of confidence. INRA's quality policy requests the validation of analytical methods when laboratories use non-standardized ones and methods used outside the scope of application of the dedicated norm. For intra-laboratory validation of quantitative analytical methods, the INRA's Quality Guidelines for research and experimental units (2013) recommends "the accuracy profile"method according to the NF V03-110: 2010 standard. This approach has the advantage of providing an overall statistical method combining trueness and precision *by* simple and graphic interpretation and allowing a rapid decision. Furthermore, it enables the determination of quantification limits, the scope of the application of the method and the estimation of uncertainty associated with the results. The process of analytical method validation allows establishing fitness for purpose by the interpretation of criteria in relation to the scientific objectives. The objective of this study was to evaluate and validate the performance of such an internally developed method applied to a set of species of terrestrial and benthic macrofauna. We expressed in particular interest in protein determination method by the bicinchoninic acid, using a commercial kit. The method was applied to 7 earthworm species, one of beetle, spider and gammarids. The validation method helped us to assess the matrix effect and to improve the steps for sample preparation and analysis. It also pointed out the importance of an experimented analyst to apply the analytical method.

TH079

Selecting optimized methods for environmental monitoring in the CCS project

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The chemical monitoring on concerned substances in the environmental samples including soils and waters is generally through analyses in the laboratories. It is axiomatic that intensive quality assurance and control (QA/QC) programs should be conducted to reduce analytical failures, specifically considering that the monitoring data plays a key role to secure the public acceptance in carbon capture and storages (CCS) projects. As the one of QA/QC activities, we've been preparing the method-selecting chart on soil and water analyses. By the methods, the confidence range of concentration and applicable matrix are various. In addition, scales of matrix interference and public susceptibility to chemical changes are different as well as concentration of each element or substances by the environmental medium. Therefore, we aim at provision of simple but secure method-selecting chart considering the matrix properties including total dissolved solids (TDS) and the expected concentrations of interfering substances. The result of this study will be an analytical-method-selecting chart for the Korean CCS project, K-COSEM (Korean CO_2 Storage Environmental Management). The substances included the chart are selected from previous results of natural analogue studies and the foreign CCS projects. The samples from CCS project can be classified into two categories by matrix: (a) soils, and (b) waters. And the chemical concentrations of each matrix from CCS can have greatly wide range due to the characteristics of each substance and released CO_2 levels. The previous monitoring data obtained from the studies on natural analogue sites and CO_2-leakage test sites also play as a yardstick as well as confidence-range of each method.

TH080

A bioassay battery for the ecotoxicity assessment of foaming agents containing anionic surfactants

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Alkylethoxysulfates (AES) surfactants are widely used in numerous industrial applications, as mechanized drill and fracking. They are present in commercial foaming products as their main components, together with different and sometimes not reported additives. In spite of their high volume utilization, very few data concerning the occurrence, fate and effects of AES on ecosystems have been published so far. The objective of this study was to evaluate the ecotoxicological effects of two commercial foaming products containing, as the main substance, the anionic surfactant sodium lauryl ether sulfate (SLES). In order to evaluate the EC_{50} values of the two commercial foaming products, they were initially subjected to ecotoxicological tests with aquatic and terrestrial organisms (Microtox test with *Vibrio fischeri*, *Pseudokirchneriella subcapitata* toxicity test and germination and growth test with *Lepidium sativum*). Then two soils with different geopedological characteristics were conditioned with the commercial foaming agents at the same concentrations used for mechanized drills and used for an experimental microcosm set-up. Soil sub-samples were collected at different times (0, 7, 14, 28 days) and soil elutriates were produced for assessing the effects of the foaming agentsboth on the previous tested organisms (*Vibrio fischeri, Pseudokirchneriella subcapitata, Lepidium sativum*) and on other two, performing the Fish Embryo Toxicity Test (FET) with *Danio rerio* and *Daphnia magna* toxicitytest. The results of the five tests were then compared with SLES residual concentrations, determined by MBAS spectrophotometric method both in the elutriates and in ASE soil extracts.

TH081

Environmental safety assessment of biomass process streams

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The bio-based production of chemicals provides opportunities for developing a safer and green chemistry. Growing biomass on contaminated soils may allow for the use of otherwise neglected land, but it may also lead to uptake and introduction of hazardous chemicals in the production chain. The (pre)treatment of biomass may lead to the formation of potentially hazardous intermediates. The presence of hazardous chemicals may not only threaten the production process itself, but it may also pose a risk to workers in the industry as well as to the environment receiving waste materials. Within the Dutch BE-Basic programme, the dRISK project (Integrated effect-based risk management for sustainable bio-based production processes) is developing tools and strategies for assessing the potential hazard of biomass streams, using bioassays based on living organisms or cells. The panel of bioassays comprises human cell lines, zebrafish embryos, daphnids, springtails and enchytraeids, and is predictive for various adverse outcomes and groups of organisms at risk. By combining chemical separation and identification techniques with the bioassays, so-called Effect-Directed Analysis, possible chemical stressors that are responsible for toxic effects can be identified. Since chemicals may be strongly embedded in the biomass, not being available and therefore posing little hazard, two extraction approaches were developed. The first one applies total chemical extraction followed by testing in the various bioassays to determine potential toxicity of biomass materials. The second approach applies passive sampling methods that extract only the available chemicals from the biomass followed by exposing the bioassays using passive dosing. The latter method is based on equilibrium partitioning and ensures that the organisms are exposed to realistic available concentrations. This also allows exposing all organisms to the same activity of potentially hazardous chemicals. This presentation will show how the tools developed in dRISK are applied to biomass obtained from the processing of verge grass, as an instrument for the safety assessment of bio-based production processes. The verge grass was harvested from road sides and as a consequence may be contaminated with metals, PAHs and other known and unknown pollutants. It therefore is an interesting substrate for bio-based production as it is unsuitable for other uses, like cattle feed, but it may also pose some of the risks outlined above.

TH082

An approach to choose the most relevant time of exposure for biomarker measurement in earthworms exposed to pesticides

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Biomarker measurements on organisms known as bioindicators are considered as good tools to assess both environmental quality and functioning. Earthworms, by their ecological functions and sensitivity, are relevant organisms to evaluate the quality of soils disturbed by human activities. Despite a great amount of experiments intended to study xenobiotics effects on the physiology of soil organisms under controlled conditions, no clear trend emerged concerning the responses of biomarkers. Owing to the high heterogeneity observed in laboratory protocols (species used as models, types and concentrations of contaminants, time of exposure, analysis methods) can even lead to contradictory results. The aim of this study was to underline the kinetic responses of biomarkers involved in earthworm physiology (defense against oxidative stress, detoxification mechanisms and energy reserves) within time. Our main hypothesis was that the conditions and particularly the time of exposure strongly influenced the results obtained in these studies. Here we provide the most relevant interval of time exposure for each biomarker. Earthworms of the species *Aporrectodea icterica* were exposed to pesticides in laboratory conditions, at realistic agronomic dose or agronomic dose x 10, alone or in a mixture. Earthworms were placed in microcosms containing contaminated soil, during periods of 2, 4, 7, 10, 14 and 21 days before biomarkers analysis. The two selected pesticide were an insecticide of the pyrethrinoids family, CYTHRINE MAX ® (Cypermethrin) and a fungicide of the strobilurin family, AMISTAR ® (Azoxystrobin), commonly applied in cereal crops. We will present the results of the kinetic responses for each selected biomarkers (catalase, glutathione-S-transferase, superoxide dismutase, proteins …), time of exposure and pesticide dose within time. We will also identify the most suitable period to their measurement.

TH083

Effect of the type of contamination and land use on soil enzymatic activities. Results of the French "Bioindicator program"

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Since many decades, enzymatic activities are used to assess the effects of land use changes, agricultural practices and soil contamination on soil functioning. A lot of studies point out contradictory results depending notably on the methodology used, as well as soil characteristics… To overcome the lack of consensus concerning indicators available to assess soil quality and functioning, the national research programme "Bioindicator" (overseen by ADEME) was set up in France to develop and assay biological indicators (2006–2012). We aimed at standardizing and emphasizing the number of biochemical measurements, and developed miniaturized colorimetric methods to obtain a great enzymatic dataset. Thirteen activities (from three laboratories) were measured within this programme, in 47 modalities of 13 experimental sites differing in terms of land use (Corine land cover), contamination type – Polycyclic Aromatic Hydrocarbures or metals – and pollution levels (Vibrisse 8^{th} decile). This dataset allows an analysis of the effect of atmospheric or added contamination gradients on the enzymatic activities. Our objectives are to point out the sensitivity of enzymes for each type of pollution and land use, to offer the most appropriate panel for a sensible analysis. Data were analysed for the all datasets, each site and each land use. Our main conclusions are as follows: enzymes are more sensitive to metallic contamination than to organic ones; enzymes are good indicators of metal bioavailability; Alkaline phosphatase and Arylamidase are the most relevant enzymes to assess the effect of soil contamination

TH084

The significance of soil microalgae and cyanobacteria to assess pesticide stresses, in agricultural soils

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Standardized soil microbial indicators, used to assess the impacts of agricultural practices and/or contaminations, lack of sensitivity and genericity against pesticide stresses, especially for herbicides. Soil photosynthetic microorganisms, mainly represented by micro-algae (chlorophyceae, xanthophyceae and diatoms) and cyanobacteria, can growth in soil surface. So, they could be an original microbiological model for herbicides risk assessment in agricultural soils. Nevertheless, their structural and functional biodiversity and the environmental factors influencing their communities are still largely unknown. Then, to improve our understanding of these photosynthetic microorganisms, we need to 1) develop biochemical and molecular methods to characterize their activities and genetic diversity and 2) analyse their responses to herbicides in agricultural soils. Several methodologies commonly used for aquatic systems were adapted to soil. Cultural approaches are still helpful to isolate edaphic species for further ecotoxicological tests and barcoding approaches. Photosynthetic pigments can provide biomass (chlorophyll-*a*) or structural (pigment diversity) indicators. Several genetic markers from aquatic studies were successfully applied on soils samples, but a current limit is the lack of databases of these specific genetic markers. Toxic effects on photosynthetic microbial biomass and diversity have been evidenced at doses close to recommended field rates, throughout field and microcosm approaches. The comparison of the responses of photosynthetic microbial communities, between different cropping systems (organic vs. conventional), highlighted a tolerance increase for some herbicides in conventional soils. Genetic diversity characterization of micro-algal and cyanobacterial communities in these cropping systems, provides further results to identify taxa relating to tolerance. Then, various genus of microalgae and cyanobacteria have been identified for their high sensitivity to herbicides. Overall, micro-algal and cyanobacterial communities showed a higher sensitivity to herbicide in soils, compared to commonly studied bacterial and fungal community. These innovative investigations demonstrate the suitable issues of soil photosynthetic microorganisms as indicators reporting non-target impacts of herbicides on soil biodiversity and functioning. Prospects for monitoring remediation of polluted soils (heavy metals, HAP) should be considered.

TH085

Use of the crustacean Gammarus fossarum in biomonitoring survey and trophic transfer of protozoa Toxoplasma gondii and Cryptosporidium parvum

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The protozoa *Toxoplasma gondii* and *Cryptosporidium parvum* are public health priorities since their oocyst form is exceptionally resistant to environment insults and can remain infectious for months or years. They are present in various watercourses as recreational, surface, drinking, river and seawater. *Cryptosporidium* oocysts were reported in a range of bivalve mollusc species but until now there is no information whether freshwater crustacea are able to accumulate protozoan oocysts. The amphipod species *Gammarus fossarum* selected in this study is widespread and common in rivers and streams of Western Europe. *G. fossarum* have an important ecological role in the trophic chain since they represent a reserve of food for macroinvertebrate, fish, bird and amphibian species. The purpose of the present study was to determine whether *Gammarus fossarum* could accumulate in their tissue *T. gondii* and *C. parvum* oocysts after experimental exposure and potential use of *Gammarus fossarum* as matrix for biomonitoring of protozoa concentration in freshwater systems. *Gammarus fossarum* were exposed to 200; 2,000 and 20,000 oocysts per gammarid and per day during 21 days followed by 5 days of depuration. DNA detection of *T. gondii* and *C. parvum* oocysts was carried out using TaqMan real-time PCR. *C. parvum* DNA was detected in gammarids in only one out of four pools for the higher concentration and after 14-day exposure. However, concerning *T. gondii*, DNA was detected after 7-days exposure with the two highest concentrations, and from 14-day exposure *T. gondii* DNA was highlighted in gammarids for all conditions. Between 0 and 14-day, oocyst number detected increased with the exposure time, then a saturation effect was observed between 14 and 21-day for the two highest concentrations. A significant dose-response relationship was observed and the maximum quantity of oocysts detected in gammarid tissues is obtained after exposure to 20,000 oocysts per organism and per day. After 5 days of depuration, *T. gondii* oocysts were still present in gammarids indicating the integrative nature of *G. fossarum*. These results show for the first time that a freshwater crustacean is able to bioaccumulate *T. gondii* oocysts suggesting that *Gammarus fossarum* is a potential effective bioindicator of protozoan contamination in biomonitoring studies. Moreover, due to their important position in freshwater food webs, *G. fossarum* could also play a role in the trophic transfer of protozoa

TH086

Snail watch to survey the transfer of PCB and PCDD/F on a polluted site converted into a photovoltaic power plant

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In 2008, a fire on a former industrial site in Saint Cyprien (France) caused a severe contamination with persistent organic pollutants (POP) to surrounding soils. About 14 hectares of pasture were consequently forbidden to agricultural use. To give a new usage to this site, a power plant was built in 2010 by Luxel SAS. This installation produces green electricity since early October 2013 and constitutes a pilot site for research on POP toxicity and degradation processes over time with bioindicators. The objective of the research program TROPE (Transfer and Risk of Organic PErsistent pollutants for snails) was to assess, thanks to active biomonitoring, the bioavailability of PCBi, PCB-DL and PCDD/F to a soil invertebrate living at the soil surface, the ubiquitous land snail, *Cantareus aspersus*. Snails were caged for 28 days on 4 grassy plots with increasing POP contamination (3631 to 34942 ng.kg^{-1} dw for ?7 PCBi, 2 to 222 ng TEQ$_{OMS2005}$.kg^{-1} dw for ?17 PCDD/F and 1.15 to 22.8 ng TEQ$_{OMS2005}$.kg^{-1} dw for ?12 PCB-DL. An additional plot highly contaminated (>400 mg.kg^{-1} PCBi and sum PCDD/F + PCB-DL > 20,000 ng TEQ.kg^{-1}) located near the studied site was also studied. Internal residues in the soft body of snails for the sum of the ?7 PCBi were from 556 to 462,124 ng.kg^{-1} fw whereas the ?17 congeners of PCDD/F varied from 0.052 to 1.18 ng TEQ .kg^{-1} and for the ?12 PCB-DL from 0.064 to 72 ngTEQ.kg^{-1}. The ratio PCDD/F / PCB-DL was from 1 to 2.6 and the ratio PCBi/PCB-DL from 4252 to 8689. Among the PCBi, congeners 101, 138 and 153 were the most accumulated pollutants. For PCB-DL, PCB105 and 118 were the most abundant whereas for the PCDD, OCDD and HpCDD were predominant in the snail tissues. Results showed that POP are bioavailable for snails and may reach high internal body concentration in highly contaminated plots. This study provides first data on the capability of land snails to accumulate various PCB, PCB-DL and PCDD/F and reveals that these soil invertebrates may be a source of POP exposure to their consumers. Two other campaigns of biomonitoring are planned in the coming years to assess the evolution of bioavailability of POP on this polluted area, unsuitable for agricultural purposes, but that has recovered an industrial value. These studies will help to sharpen our understanding of the PCB impact on the ecosystems.

TH087

Evaluation of an endocrine disruptor (Vinclozolin) in the benthic organism Chironomus riparius (Diptera)

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The dicarboxiamide Vinclozolin (Vz) is a widely used fungicide for agricultural purposes. Because of this, it is found as a residue in the environment, detected in underground water, lakes, rivers, and seawater. Most of the toxicity studies have been focused on vertebrates showing antiandrogenic activity, however, few studies have investigated their effects on invertebrates, which are key organisms in aquatic ecosystem maintaining. In this work we have analyzed the effects caused by Vz on fourth instar larvaeof *Chironomus riparius*, a dipteran used frequently as reference species in ecotoxicology. Alterations that exposure of the larvae for 24 and 48 hours causes on several genes related with the endocrine system and the detoxification pathways of this insect were evaluated using Real-Time PCR. Two of them, *Disembodied* and *Cyp18a1*, are related with the metabolism of ecdysone, one of the main hormones of insects involved on development. *Membrane Associated Progresterone Receptor*, the coding gene for an orphan receptor, and *Forkhead Box O*, a gene which produces a protein involved in the insulin response pathway, were also analyzed. On the other hand, *Cyp450G* and *GSTd3* transcriptional activity was studied to analyze if they are involved in the detoxification process of Vz. The results show a significant alteration of transcription activity of some of these genes indicating that the presence of Vz affects the cell processes in this organism. The results obtained show the ability of Vz to up-regulate hormonal related genes in *C. riparius* larvae after exposures and establish the first evidence of its endocrine disruptive effects in insects. This work was supported by the Plan Nacional de Investigación Científica, Desarrollo e Innovación Tecnológica (Spain), grant CTM2012-337547 from the Ciencias y Tecnologías Medioambientales program. M.A. is the receiver of a predoctoral contract Ministry of Economy and Finance (BES-2013-064041).

TH088

Formaldehyde Chemical Substance Flow Analysis in South Korea

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A chemical substance is any material that has a definite chemical composition (Hill et al. 2005). Currently chemical substances and materials are widely used in all kind of industry and household, almost everywhere and in everything. On the other hand, these chemical substances and materials have important issues such as risk related with hazardous and toxic in their life cycle from manufacturing and use to the end of life. Also historically, many chemical accidents and industrial disasters were occurred in the world. Up to date, therefore, related regulation and assessment tools (e.g., Regulation on Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), risk assessment, health effect of chemical exposure) are widely applied for chemical substances and materials. There have been some studies on the guiding principles of chemical accident prevention, preparedness and response. However, the study on chemical substance flow analysis in country level and its application was not well conducted. In this study, therefore, formaldehyde (CH_2O), which is used mainly to produce resins used in particleboard products and as an intermediate in the synthesis of other chemicals was selected. Formaldehyde is used predominantly as a chemical intermediate. It also has minor uses in agriculture, as an analytical reagent, in concrete and plaster additives, cosmetics, disinfectants, fumigants, photography, and wood preservation (US EPA, 1988). The purpose of this study is quantifying, analysing and mapping of the formaldehyde substance flows by using material flow analysis (MFA) method in South Korea. Our result shows that the number, location of manufactures using formaldehyde as well as formaldehyde's flow (import, export, stocks and flows) in Korean industrial system (between industries including companies). Finally the result of this study can support for chemical accident prevention system and sustainable chemical management in South Korea.

TH089

Biological effects of different pesticides and their mixtures on Eisenia andrei: laboratory experiments and field studies using caged earthworms

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Pesticides are harmful to specific target organisms; however, selectivity is never absolute and therefore pesticides represent a risk also to non-target organisms. Most of the studies using model organisms in ecotoxicology have focused on the evaluation of high-level endpoints (i.e. mortality and reproduction) that provide information about possible effects at organism/population level; data concerning sublethal effects have been rarely described. In this study we evaluated the toxicity of different pesticides widely used in agriculture on the earthworm *Eisenia andrei* by simulating in the laboratory the processes carried out in the rice- and vine-fields. For this purpose, a set of biomarkers able to highlight the alterations of the health status of the organisms from the most sensitive molecular changes to whole-organism responses was employed. The results demonstrated that all the studied compounds at concentrations recommended by the manufacturers did not affect the worm survival but provoked relevant stress and genotoxic effects on 28 d exposed animals. To verify the results of this laboratory study, we also realized field studies. To this end, a method was developed to maintain the earthworms in field for 10/28 d. The animals caged in the sites of rice (during the dry period) and vine cultivation treated with the pesticides (at the doses used in laboratory experiments) survived. However, also in these animals, significant effects on sublethal endpoints (such as lysosomal membrane stability, Ca^{+2}-ATPase activity and mitochondrial functionality) were observed. In both crops, the results highlighted genotoxic effects as demonstrated by the increase of the level of the DNA damage and of the micronuclei frequency.

TH090

Application of bioassays with Enchytraeus crypticus to evaluate the effect of biochar in metal bioavailability

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Mining activity results in the accumulation of waste scattered in the landscape. When contaminants contained in these wastes are mobilized, the risk of pollution of surrounding areas increases, degrading soil quality and causing serious problems to human health. The main objective of MINETOX project is to study the effect of biochar in metal availability of different mine wastes over time by means of the physicochemical and ecotoxicological characterization of the treatments. In this study, two mine wastes (acid and basic) were amended with two different types of biochar (one from pruning remains and other from sewage sludge) separately, in order to study their effect on metal bioavailability and to modify their potential environmental impacts. Since the application of the amendment was expected to result in metal immobilization in soils, the efficiency was tested both in mine wastes and mine waste pore waters. Mine wastes were amended with 6% of biochar, and pore waters were extracted at the beginning and the end (after 6 months of incubation) of the experiments for all treated and untreated wastes. Accumulation and toxicity of treated and untreated wastes in the potworm *Enchytraeus crypticus* were investigated in mine waste pore water solution embedded in an inert quartz sand matrix. Additionally, treated and untreated wastes were mixed with uncontaminated Lufa 2.2 reference natural soil to obtain waste concentration of 100, 50, 25, 12.5, 6.25 and 3.13% (w/w), and survival and reproduction was observed in all treatment at each dilution concentration. Preliminary results show than organisms were more affected when exposed to mine wastes than when exposed to the pore water solutions. The latter could indicate that when exposed to mine wastes the organisms could be still affected by the fraction of metals immobilized in the mine wastes. Toxicity seems to decrease when biochar was added to mine waste but differences between two types of biochar were not significant. In this study, we evaluate the efficiency of different amendments from a double perspective, taking into account the two different exposure pathways (via pore water and via soil ingestion).

TH091

Stratification of soil arthropods in top soil layers

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The persistence of pesticides in soil is well studied. Their distribution depends on their physical and chemical properties together with the soil characteristics. Studies have shown that traces of pesticides can be found in soil up to several years and down to depths over two meters. Little is known on the stratification of the soil arthropods in the top soil layers and even less about the effects of pesticides on them. Therefore, our overall aim is to study these communities and their response to pesticide exposure. An ongoing pilot study, started in May 2015, aims to identify the arthropods groups found in the top 40 cm of soil and record their seasonal activity. Soil arthropods were sampled with a tubular trap ("slide trap") specifically designed for this aim, at four depths: 0-10 cm, 10-20 cm, 20-30 cm and 30-40 cm. Each tube has approximatively 70 holes of 5 mm diameter evenly distributed at the target depth for a maximized stratified sampling. To collect the arthropods a funnel and a jar, filled with 50 ml of ethyl-glycol, were attached at the bottom of the tubes. The traps were setup in May 2015 and are checked every two weeks. Results of the first six months show that adult beetles and their larvae can be found up to 40 cm depth, and their vertical migration appears to be influenced by the environmental conditions. Species of Diplura are mainly found at 20-30 cm depth. Individuals of the subphylum Myriapoda have a split stratification, with high numbers in the layers of 0-10 cm and 20-30 cm. Remarkably species of Pauropoda and Pseudoscorpions show up frequently in layers deeper than 20 cm. Full taxonomic list for all groups and a detailed model of the slide traps used will be presented with the poster. Presently studies are designed to look at the arthropods communities in the first 10 cm and might overlook the effects of pesticides on taxa such as Diplura and Myriapoda, groups that are more abundant in deeper layers. Our study will bring valuable information on how these communities respond to pesticides in deeper layers missed in previous in situ studies. Also it will show if and how the environmental conditions influence the response.

TH092

Using in situ assays with two freshwater producers to assess effects of wildfires on aquatic systems

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Portugal is the European country where forest fires devastate more area, with an average of 144 000 ha per year in the last decade, and a tendency to increase both in frequency and area. Wildfires constitute a concerning environmental problem once they have adverse and diversified effects impacts in the terrestrial, atmospheric and aquatic compartments. It is known that wildfires affect aquatic systems by altering its physical and chemical proprieties through the input of pyrolytic substances, such as polycyclic aromatic hydrocarbons (PAHs), and metals associated to ash/soil loads from the surrounding burnt areas. The majority of the available studies regarding the effects of wildfires on aquatic biota have been performed by using laboratory single species bioassays. Once *in situ* exposition enable to assess more realistic environmental multi-stress conditions and their fluctuations, in situ assays using two aquatic producers' species –the microalgae *Raphidocelis subcapitata* and the macrophyta *Lemna minor* - were performed. A recently burnt area was selected near Miranda do Corvo (Portugal) and five sites were selected to conduct the assays: two in the main water course of the watershed - Ceira river, being one located upstream (RUS) and the other downstream (RDS) the burnt area; two in tributary streams within the burnt area (SUS and SDS); and one in a stream located in an adjacent non-burnt area (CS). The assays took place at the time of the first major rainfall events and lasted for 13 days. Personalized test chambers were elaborated, allowing floatage and luminosity entrance for both tested species. *R. subcapitata* was immobilized in alginate beads while *L. minor* leaf colonies were tested freeform. The cellular concentration of microalgae in beads and the initial number of fronds and weight for *L. minor* were determined in the beginning and in the end of the assay, allowing to calculate the growth rate. The present results showed an inhibition in the growth rates of both species when exposed to the most impacted sites (RDS, SUS and SDS) comparatively to the reference sites (RUS and CS) . Hence, the *in situ* bioassays revealed to be effective to discern toxic effects on aquatic primary producers due to diffuse contamination from wildfires.

TH093

Off-site environmental impacts of wildfires: Assessment of the toxicity of post-fire runoff on freshwater aquatic species

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Wildfire is a major disturbance of forests worldwide, posing an important threat to life, human goods and natural resources. Interestingly, wildfires have an important role in contaminants production and mobilization, such as polycyclic aromatic hydrocarbons (PAHs) and trace elements. For instance, these contaminants could be mobilized during a wildfire from ashes and sediments loads and may eventually reach the nearby aquatic systems by surface runoff. In this regard, wildfires represent a relevant diffuse source of PAHs and trace elements to aquatic systems that has, so far, been poorly investigated, in particular, their potential toxicity on aquatic biota. The current study aims to mitigate such lack of knowledge for PAHs and trace elements, a well-recognized persistent toxicants with potential harmful impacts on the environment and on human health. To address the potential ecotoxicological effects posed by such compounds and their mixtures, the present work was focused on their effects in the following aquatic species: bacteria (*Vibrio fischeri*), the algae (*Raphidocelis subcapitata*), the macrophyte (*Lemna minor*) and the invertebrate (*Daphnia magna*). These species were exposed to different dilutions (12.5, 25, 50, 75 and 100%) of post-fire runoff and surface water from the burnt catchment outlet. All the samples were collected during the first rainfall events after the wildfire. The concentration of the fifteen PAHs identified by USEPA as priority contaminants were analysed by gas chromatography-mass spectrometry (GC-MS) and the trace elements (V, Mn, Co, Ni, Cu, As, Cd and Pb) by inductively coupled plasma-mass spectrometry (ICP-MS). The endpoints tested in the ecotoxicological assays were: luminescence inhibition for the *V. fischeri*, growth inhibition for the *R. subcapitata* and the *L. minor*, and immobilization for the *D. magna*. Lethal and sub-lethal dose response curves were determined as well as the corresponding endpoints (NOEC, LOEC, EC_{50} and EC_{20}). The preliminary results allowed to discriminate the off-site effects of wildfires on distinct aquatic species. Moreover, it allowed to determinate the threshold concentrations of PAHs and trace elements that can be considered at low/high risk for aquatic biota and therefore, the aquatic systems. This information is of crucial importance to predict the risks and effects of the surface water pollution by recently burnt areas and the propagation of toxic effects from the lower to the upper trophic levels.

TH094

Biofuels and their aquatic toxicity - Biotesting 3-methyltetrahydrofuran

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The rapidly growing industrial sectors in many developing countries, such as China or India, require an increasing volume of fuel for the transport sector. In parallel, environmental concerns regarding petroleum based fuels and the request for independency on oil result in a growing demand for renewable energy sources such as biofuels. However, a release into the environment increases with a rise in biofuel production and consumption. Therefore, a comprehensive assessment of the environmental hazard potential is aggravated by the lack of ecotoxicological data. Ecotoxicological bioassays can be used for the detection of potential hazards for aquatic ecosystems at a very early stage of the development. This study is focused on the ecotoxicological investigation of the potential biofuel 3-methyltetrahydrofuran (3-MTHF) in aquatic systems. To evaluate the hazard potential of this new potential biofuel for the environment 3-MTHF has to be tested in different aquatic testsystems monitoring different levels of organisation, such as acute toxicity and embryo toxicity using the fish embryo toxicity (FET) test with *Danio rerio* (OECD 236), the acute immobilization assay with *Daphnia magna* (OECD 202) and algae growth inhibition test with *Desmodesmus subspicatus* (OECD 201). Additionally an *in vitro* micronucleus-test with Chinese hamster V79 cells (ISO 21427-2) was performed to investigate the genotoxic potential of the fuel candidate. Differences in toxic potencies of 3-MTHF compared to other fuel candidates can be used to identify the least toxic biofuels and focus further research on these candidates. An early investigation of biofuels can decrease the risk for the environment and, thus, reduce the potential of these new fuels to become a source of contamination for aquatic systems.

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TH095

Occurrence of four potentially SVHC-classified semipolar PAHs in selected environmental matrices and their ecotoxicological impacts

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Semipolar polycyclic aromatic hydrocarbons (heterocyclic PAHs or PANHs, PASHs and PAOHs) are often found in UVCBs (chemical substances of unknown or variable composition, complex reaction products and biological materials) from coal and crude mineral oil and derived products in association with homocyclic PAHs (Schwarz et al., 2014). In spite of the fact that there are some indications of toxic properties comparable to their homocyclic analogues, the toxicological characterization of semipolar PAHs is often still insufficient. Identification and characterization of substances of very high concern (SVHC) is one of the key components of the REACH (registration, evaluation, authorization and restriction of chemicals) Regulation of the European Union. Annex XIII of the REACH Regulation defines the properties of SVHC. Substances with persistent, bioaccumulative and toxic properties (PBT) are of very high concern for the environment and can be regulated by authorities. Within a joint research project the German Environment Agency (UBA) and the Biochemical Institute for Environmental Carcinogens (BIU) investigated the ecotoxicological properties of four semipolar PAHs potentially identified as SVHCs: - Benzo[*b*]naphtho[2,1-*d*]thiophene - Dibenz[*a,j*]acridine - Benzo[*b*]naphtho[1,2-*d*]furan - 7*H*-Dibenzo[*c,g*]carbazole The ecotoxicological investigations were carried out at the UBA's ecotoxicological laboratory in accordance to OECD test guidelines using aquatic organisms from different trophic levels: green algae (*Desmodesmus subspicatus*), crustaceans (*Daphnia magna*) and fish embryo (*Danio rerio*). At the BIU semipolar PAHs were prepared in high purity and water solubility of each compound was determined in the used test media and at the relevant test temperatures. The results obtained for the PAH's ecotoxicological potential on the three investigated aquatic species are presented. In addition, PAH levels found in blue mussels (*Mytilus edulis* L.) collected at several locations along the German coastline as well as PAH levels found in fresh kale (*Brassica oleracea* var. *sabellica* L.) and certified harbour sediment (BCR-535) are reported. Reference: Schwarz, M., Behnke, A., Brandt, M., Eisenträger, A., Hassauer, M., Kalberlah, F., Seidel, A. (2014) Semipolar polycyclic aromatic compounds: Identification of 15 priority substances and the need for regulatory steps under REACH regulation. Integr. Environ. Assess. Manag. 10: 415-428.

TH096

Oral Bioavailability Study of PAHs in Coal Tar/Coal Tar Pitch Clay Pigeon Target Fragments from Range Sites

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An *in vivo* oral bioavailability study was performed to determine the relative bioavailability of PAHs in clay pigeon target fragments at military range sites. These fragments, which are mixed with native soil, are composed of PAHs in a site-aged coal tar/coal tar pitch and limestone matrix. It was expected that the fragment matrix would reduce the oral bioavailability of PAHs compared to that seen in animal studies using pure benzo(a)pyrene in solvents. To test this hypothesis, an oral bioavailability study was performed with female B6C3F1 mice (8 mice per dose group) fed soils or soil extracts at a rate of 5% in the diet for fourteen days. Four test soils contained 0.5, 4.5, 28 or 183 mg/kg benzo(a)pyrene (BaP). BaP concentrations in diets were 0.04, 0.39, 2.55, and 19.17 mg/kg. A high resolution mass spectrometry method was developed to detect low levels of the following PAH metabolites in mouse urine: 3-hydroxy-benzo(a)pyrene, 9-hydroxy-benzo(a)pyrene, 3-hydroxy-benz(a)anthracene, and 3-hydroxy-chrysene. The fractions of total dose excreted in the urine were compared for the soil and the soil extract groups to derive Relative Bioavailability Factors (RBAFs) for use in risk assessment. The RBAFs were 0.20 for BaP, 0.23 for benz(a)anthracene, and 0.28 for chrysene.

TH097

Dermal Absorption Study of PAHs in Coal Tar/Coal Tar Pitch Clay Pigeon Target Fragments from Range Sites

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An *in vitro* dermal absorption study with human skin was performed to determine the dermal absorption of PAHs in clay pigeon target fragments at military range sites. These fragments, which are mixed with native soil, are composed of PAHs in a site-aged coal tar/coal tar pitch and limestone matrix. It was expected that the matrix would reduce the dermal absorption of PAHs compared to that seen in animal studies using pure benzo(a)pyrene (BaP) in solvents. For human health risk assessment, USEPA assumes that 13% of administered PAHs are absorbed based on an *in vivo* study in rhesus monkeys by Wester et al. (1990). This study evaluated dermal absorption by spiking *unaged* soil samples with ^{14}C-BaP and testing the spiked soil immediately. Several published studies showed that aged, weathered PAHs have lower dermal absorption compared to freshly spiked soil samples. To test this hypothesis, an *in vitro* study was performed in Franz diffusion cells with split-thickness fresh-frozen human skin samples from male and female donors aged 23-65 years. Test articles were applied to skin for 24 hours, then washed off. Collection of receptor fluid samples continued for a total monitoring period of 72 hours. Skin samples were stripped five times with tape to remove the stratum corneum and any remaining surface soil. PAHs were analyzed in the dermis and in the surfactant-containing receptor fluid by high performance liquid chromatography with fluorescence detection to a detection limit of 100 ng/mL to define absorbed dose. Test articles included three site soils with BaP concentrations of 8, 51 and 383 ppm. Dermal absorption of BaP and six potentially carcinogenic PAHs was less than 1%. A freshly spiked control sample gave 9% absorption for BaP.

TH098

Ecological Risk Assessment of Polycyclic Aromatic Hydrocarbons in Water, Sediment and Dominant Fish from Designated Zones of the Lagos Lagoon, Nigeria

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The ecological risk assessment (ERA) of priority pollutants such as Polycyclic Aromatic Hydrocarbons (PAHs) are imperative due to their cascading biological effects along the food chain in the aquatic environment. In this study, the levels and ecological risk assessment of sixteen (16) priority PAHs were evaluated in water, sediment and dominant fish species sampled from designated anthropogenic-impacted zones (Ilaje, Iddo, Atlas cove and Apapa) of the Lagos lagoon in the wet and dry seasons from 2012 to 2014. PAHs in the samples were analyzed using GC/FID. Indices such as Effects Range Low/Effect Range medium (ERL/ERM), Threshold / Probable Effects Level (TEL/PEL), sediment and fish pollution classification based on sum PAHs and Bioconcentration Factor (BCF) were utilized for the ERA. The results showed that the dominant fish species were *Sarotherodon melanotheron* (Black-Chinned Tilapia), *Gerres melanopterus* (Gerres), *Liza falcipinnis* (Sickle-fin Mullet) and *Pseudolithus elongatus* (Bobo Croaker) at the Ilaje, Iddo, Atlas cove and Apapa zones respectively. The average sum 16 PAHs (∑PAHs) and range were 515.58µg/L (194.94 (Ilaje, wet season) - 1006.49μg/L (Apapa, wet season)), 739.45μg/kg (301.81 (Ilaje, wet season) - 1290.16μg/kg (Apapa, wet season)) and 19.78μg/kg (8.80 (*S. melanotheron*, dry season) - 26.10μg/kg (*G. melanopterus*, dry season)) in water, sediment and fish respectively. Naphthalene was dominant in water and sediment samples while phenanthrene, anthracene, fluoranthene and pyrene were predominant in the fish species across the zones and seasons. ERA of PAHs in the sediments showed that biological effects will occur frequently at the Apapa zone and occasionally at all other zones based on the levels of naphthalene, acenapthylene, acenaphthene, fluorene and benzo(a)anthracene in both seasons. Apapa zone was highly polluted while other zones were moderately polluted in both seasons based on ∑PAHs in the sediments. Fish species from all the zones were minimally contaminated in both seasons except *S. melanotheron* based on ∑PAHs in fish. BCF results showed that PAHs did not bioconcentrate in the fish species except for pyrene with a BCF value of 2.28 in *G. melanopterus*. The study recommends that the specific PAHs identified as ecological risk factors in the Lagos lagoon sediments should form a basis for the establishment of environmental quality standards for PAHs in Nigerian coastal waters as well as their inclusion in ecomonitoring programmes.

TH099

Water quality assessment of five cascading reservoirs of Iguaçu River through multi-biomarker approach in fish

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The Iguaçu River, located at southern region of Brazil, has great socioeconomic and environmental importance due to its high endemic fish fauna as well as its ability to generate hydroelectric power and the potential to supply water for human use. To date, no previous study has reported an integrated evaluation of chemical analysis and biological responses in Iguaçu River, which is considered ideal for a better environmental risk assessment. The local environmental agency describes water quality improvement along the river course, since there is intense discharge of pollutants in the river's source. For the current study, three different Brazilian fish species (*Astyanax bifasciatus, Chrenicicla iguassuensis* and *Geophagus brasiliensis*) were captured for a multi-biomarkers approach analysis in five cascading reservoirs of Iguaçu River. Chemical analysis in water, sediment and muscle indicated high levels of metals in all reservoirs. Chromium (Cr) levels in water were detected above the permitted concentrations set by governmental agencies. High levels of polycyclic aromatic hydrocarbons (PAHs) were also detected in fish's bile for the three different species analyzed. An ELISA kit produced for the native species *Geophagus brasiliensis* detected vitellogenin expression in male fish from the five reservoirs. Integration of the data through FA/PCA analysis demonstrated the poorest environmental quality of the reservoir farthest from river's source, which is the opposite of what is reported by the environmental agency. The high levels of metals and PAHs in the five reservoirs of Iguaçu River associated with the biological responses of fish show the impacts by anthropic activities to this area and not confirm a gradient of pollution from the source toward Iguaçu River's mouth.

TH100

Sediment quality assessment in Ho Chi Minh City canals.

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Ho Chi Minh City metropolitan region is the largest urban and industrial hub of South Vietnam, with more than 6 million inhabitants and a large number of big factories and artisanal plants. Located in the Mekong delta, the area is characterized by a complex system of canals that play a vital role for the region and its inhabitants. They act as reservoirs for water run-off and drainage and they are used for transportation and as a source for natural resources. Due to public health concerns an important sanitation program has been undertaken in the last decade for the reduction of wastewater pollution and flooding. At present, the main canals are recovering after clean-up and clean waters have attracted fish again, with increasing stocks thanks to repopulation initiatives. In 2015 we carried out a pilot study to assess the quality of recent sediments from Ho Chi Minh City canals. Surface sediments were collected from 6 canals, 4 of them located in the metropolitan area and 2 from suburban areas. Sediments were subject to physico-chemical and ecotoxicological characterisation, the physico-chemical characterisation consisting of the quantification of trace metals and organic micropollutants and the ecotoxicological assessment through the use of existing chemical quality guidelines and standard sediment toxicity tests. The results show that the sediments from the canals from both the metropolitan and suburban areas accumulate a mixture of contaminants with concentrations of several PAHs and PCBs above the corresponding thresholds for effects to benthic organisms. Several pesticides were quantified in the sediments but the highest concentrations were measured for permethrin in the case of the sediments from the metropolitan area and for bifenthrin in sediments from suburban areas (3692.9 ng/g dw and 477.6 ng/g dw, respectively). Conspicuous concentrations at the μg/g order were found for the fragrances galaxolide and OTNE whereas nonylphenol, triclosan and the UV filters octocrylene, 2-EHDP and EHMC were measured at all canals at concentrations of several tens and even hundreds ng/g. The results of the chemical analyses are corroborated by the different levels of toxicity to crustaceans although no or slight toxicity was observed to rooted macrophytes in laboratory toxicity tests.

TH101

The Impact of Surrounding Land Use and Site History on Metal Concentrations and Bioaccessibility in Canadian Urban Parks

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To assess the potential risk associated with exposure to metal contaminants in Canadian urban green spaces, surface soil samples taken from exposed areas with high potential for human soil contact at various parks and playgrounds were analyzed for total metals, pH, TOC and metal bioaccessibility. The green spaces were selected from Halifax (10), Saint John (11), Fredericton (11), Ottawa (8), Toronto (17), Montreal (20), Regina (8), Winnipeg (8), Edmonton (17) and Victoria (22). Arsenic, Cr, Cu, Pb or Zn concentrations in 19 out of the 134 soil samples analyzed exceeded the Canadian Council of Ministers of the Environment (CCME) soil quality guideline for residential/parkland use. Apart from the influence of naturally occurring bedrock on As, soil metal concentrations were influenced by the proximity of the parks to industrial activities (e.g., metal recycling), heavy traffic corridors and former contaminated sites. Arsenic concentrations were particularly elevated in Halifax , Saint John and Fredericton. The elevated concentrations in these three cities reflected natural geological processes since some areas in the provinces these cities occur have high natural As concentrations that exceed human health risk-based guidelines due to bedrock and natural soil enrichment. The highest Pb concentrations (were detected in parks located near heavy traffic corridors while elevated Cu, Cr and Zn levels were associated with parks situated either on former industrial sites or near current metal related industrial sites. The mean metal bioaccessibility were As (24%), Cd (80%), Cu (39%), Pb (61%), Ni (17%) and Zn (32%). Based on the total metal concentrations, soil properties and bioaccessibility data the risk associated with exposure to metals in soils in most of the urban parks studied were deemed fairly low except Pb.

TH102

In vivo and in situ methods for assessment of the implication from a municipal wastewater treatment plant for the receiving stream

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Micropollutants, like pharmaceutical residues, in municipal wastewater are not sufficiently removed by secondary and tertiary treatment methods in wastewater treatment plants (WWTPs). As a consequence, complex mixtures of micropollutants discharge into the receiving streams and may cause various effects on aquatic biota, like fish and macroinvertebrates. To decrease, or even fully prevent the release of micropollutants into the environment qarternary treatment methods, like ozonation, are currently tested in several WWTPs in Switzerland and Germany. In the course of the DemO_3-Project, a full-scale ozonation is planned to be built at the WWTP Soers in Aachen, Germany. A crucial part of this project is the evaluation of the ecological and chemical state of the receiving stream, the River Wurm. To be able to determine the impact of the ozonation on the state of the stream, the evaluation is conducted before and after the implementation.The present study focused on the status quo of the River Wurm and aimed to determine the ecotoxicological implication from WWTP Soers for the river. In order to assess occuring effects, water samples were taken after three treatment steps at the WWTP and at three sampling sites along the river: upstream of the rain spillway basin, downstream of the rain spillway basin and downstream (approx. 2 km) of the WWTP. These samples were tested as both native samples and extracts on embryos of *Danio rerio* (FET). Further, an*in situ*bioassay on feeding rate inhibition with encaged *Gammarus pulex* was conducted for 7 days. The gammarids were exposed at the mentioned sampling sites in the river and an additional site immediately after the outlet of the WWTP.The first results of the FET show no impairment at all for the river samples, whereas, as expected, the embryos were heavily affected by the WWTP samples. The highest ecotoxicological potential was found in the inlet, where a 100 % mortality was found in the two highest concentrations, followed by a high number of sublethal effects in the lower concentrations. The first results of the feeding rate inhibition test indicate a decrease of ca. 30 % in feeding rate downstream of the rain spill basin, while the highest feeding rate was found 2 km downstream the WWTP. The lowest feeding rate or the highest inhibition is expected to be found immediately after the WWTP outlet. To support and strengthen these first findings, further tests are needed.

TH103

Mechanism-specific evaluation of a waste water treatment plant and its downstream river Wurm

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The European Water Framework Directive aims to achieve a good chemical and biological state of all surface waters until 2015. However, the good biological state is only reached by around 20 % of the German surface water bodies. One reason might be the release of a variety of anthropogenic contaminants by waste water treatment plants (WWTPs). It could occur that these substances are not sufficiently eliminated via the conventional cleaning processes. Those chemicals can lead to adverse effects. The federal level as well as the state level policy demands the elimination of trace components in the effluent of WWTPs by a progressing treatment stage. There are several advanced treatment processes, one example is the ozonation. Based on that a commercial-scale plant should be installed to the WWTP "Aachen Soers" associated to the project DemO_3AC. This WWTP is located in the city of Aachen and releases its effluent to the River Wurm. At medium and low water the river runs around 70 % treated waste water. To give evidence how the ozonation influences the ecological state of the River Wurm and the effluent of the waste water treatment plant the status quo of the river as well as of the WWTP are evaluated. Before testing those samples a sample pretreatment was conducted via a solid phase extraction over Oasis HLB cartridges with Methanol and Dichloromethane. For each sample site 2.5 l of water were filtered and extracted. After evaporating the samples and drying under a constant nitrogen stream the extract was dissolved in Dimethylsulfoxide to reach a final concentration of 2500 fold. Those samples are tested in several mechanism specific bioassays. Based on the results of the MTT assay – an assay by which the cytotoxicity of samples for different cell lines can be tested – the ERα CALUX assay evaluating the ERα Receptor mediated effect as well as the H295R assay evaluating the human steroidogenesis are conducted. To get information on the genotoxic effect of the samples the AMES assay with the bacteria strains TA 98 and TA 100 is carried out. First results for the AMES assay, the MTT assay as well as for the CALUX assay are generated but not yet finally evaluated. Those results will be available for the conference. Based on the results of the evaluation of the status quo a test battery for waste water evaluation should be developed.

TH104

Spotting opportunities amidst environmental degradation: Indigenous soil bacteria of mining sites.

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Diverse anthropogenic activities have resulted to heavy metal pollution and hence environmental degradation, posing huge challenges on soil and water resources as well as human health safety. Indigenous microbes from these degraded environments have proved to be valuable eco-friendly tools in the restoration of such environments. This study investigated the heavy metal resistance profile of bacteria for possible bioremediation strategy. Bacteria were isolated from soils collected from three active gold and gemstone mine sites in South - western, Nigeria. These were identified using 16S rRNA gene analysis. Isolates were exposed to five heavy metal concentrations viz: mercury (Hg), cadmium (Cd), lead (Pb), copper (Cu), nickel (Ni), aluminum (Al), manganese (Mn), zinc (Zn), iron (Fe) and chromium (Cr) supplemented into broth media. Growth absorbance was recorded by Micro well 96 well plate reader at 520 nm wavelength every 30 minutes for 48 hours. Minimum inhibitory concentrations (MICs) were then determined from the growth curves generated. Identified bacterial isolates were: *Lysinibacillus macroides* (LM), *Achromobacter spanius* (AS), *Bacillus kochii* (BK), *Klebsiella pneumoniae* (KP), *Pseudomonas mosselii* (PM), *Pseudomonas nitroreducens* (PN) and *Bacillus cereus* (BC). All isolates showed resistance to Pb, Ni, Mn, Fe and Al to highest concentrations >300, 100, 600, 600 and 100 ppm respectively while PN, KP, BK, AS and BC showed resistance to Zn (>300 ppm concentration). A 25 ppm Hg, Cr >100 ppm and Cd >50 ppm were tolerated by PN, PM and KP, while BK, AS and BC tolerated higher concentrations at Hg (>50 ppm), Cr (>150 ppm) and Cd (>100 ppm). *Pseudomonas nitroreducens*, PM, BK, AS and BC expressed tolerance to Cu >200 ppm concentrations. The heavy metal concentrations to which these bacteria showed resistance exceeded set permissible limits for contaminated soils globally. These results therefore suggest these bacteria isolates as potential bioremediation candidates for restoration of heavy metal polluted environments.

TH105

Bioremediation of oil-polluted soil in Caspian Sea coast by biodegradation bacteria

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The quality of life on Earth is linked inextricably to the overall quality of the environment. Bioremediation is an option that offers the possibility to destroy or render harmless various contaminants using natural biological activity. In this research 7 genera of biodegradation bacteria which are important in bioremediation process were isolated and identified. We had been used the soil sample of Amir Abad Chamkhaleh (the coast of Caspian Sea). Adding the soil samples to MSM (Mineral Salt Media) culture for isolating the biodegradation bacteria and then these bacteria inoculated to some media which had hydrocarbon as a carbon source and after isolating with alternative culture these bacteria were considered and characterized based on morphological biochemical and fermentation tests. According to the Bergy manual of systematic bacteriology these bacteria were identified. Although, in this study we had been used of plasmid extraction method for considering that if biodegradation genes exist in the plasmid or linear DNA .

TH106

Assessment of the potentail of biochar and coal tailings as atrazine sorbents

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The intensive application of organochlorine pesticide results in the contamination of ground and surface waters. The objective of this study was to investigate the potential of carbonaceous matrices in the treatment of water contaminated by the herbicide atrazine (ATZ) by evaluating the sorption capacity of ATZ by biochar and nitrated coal tailing samples. Adsorption of ATZ was conducted both on the original coal tailings as on the nitrated samples. The nitration was conducted at 70°C under reflux (sohxlet) employing 25% HNO_3solution. In centrifuge tubes of 50 mL, 0.3 g of adsorbent were weighed and 30 mL of ATZ solution were added, at concentrations of 0, 1, 2, 4, 5 and 10 mg ATZ L^{-1} ($CaCl_2$ 0.01 mol L^{-1} medium). After 24 hours stirring in the dark, the suspension was centrifuged (2500 rpm for 15 minutes) and the supernatant was filtered and stored in dark bottles. Aliquots of the supernatant were taken for determining the concentration of atrazine in equilibrium in the solution by ultraviolet-visible spectroscopy (UV-Vis, UV-160 Shimadzu) at 222 nm. In all tested sorbents the tadsorption was reversible, indicating that these materials can be reused. The nitration of coal tailings improved their adsorption capacity and this behaviour was related to a change of the surface morfology rather to the increase of surface hydrophilicity. Nevertheless the adsorption capacity of nitrated samples was inferior to that of activated charcoal.

TH107

Butadiene Chemical Substance Flow Analysis in South Korea

J. Kim, Inha University; J. Kim, University of Technology of Troyes / Department of Humanities Environment Information Technology CREIDD; J. Moon, Y. Hwang, Inha University

Currently chemical substances and materials are widely used in all kind of industry and household, almost everywhere and in everything. On the other hand, many accidents related with chemicals are also occurring in the world. In South Korea, the accidents related with chemicals are getting increased from 7 cases in 2006 to 104 cases in 2014 (MOE, 2015). Along with establishment of chemical regulations such as EU REACH and Korea REACH - The Act on the Registration and Evaluation of Chemicals), many studies should be conducted for sustainable chemical substance's safety management and prevention of the chemical accidents. In this study, butadiene (1,3-Butadiene, C_4H_6, CAS NO: 106-99-0) in the important chemicals was selected. The butadiene is flammable, colorless gas with a mild aromatic odor and it is highly reactive. Butadiene is soluble in alcohol and ether, insoluble in water and polymerizes readily, particularly if oxygen is present. One major use of butadiene has been in the making of synthetic rubber. Butadiene is also used extensively for various polymerizations for plastics manufacturing. The purpose of this study, therefore, is to quantify, analyse and make a flow map of the butadiene by applying substance flow analysis (SFA). Our result shows that the amount consumption in each company, location of manufactures using butadiene as well as butadiene's flow (import, export, stocks and flows) in Korean industrial system (between industries including companies). Finally the result of this study can support for chemical accident prevention system and sustainable chemical management in South Korea.

TH108

A Study on Hydrogen Fluoride Substance Flow Analysis : In Case of the Chemical Industry in South Korea

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Up to date, many chemical accidents were occurred in the world. We know that recently there was a big chemical explosion accident and its expose of toxic chemicals in Tianjin, China. According to the Korean Ministry of Environment, the number of chemical accidents was increased 7 cases in 2006 to 104 cases in 2014. Therefore, it should be considered and conducted many studies on sustainable chemical substance's safety management and prevention of the chemical accidents as well as establishment of related chemical management regulations. In this study, we selected and studied about hydrogen fluoride (the chemical formula HF, CAS NO: 7664-39-3). This colorless gas or liquid is the principal industrial source of fluorine, often in the aqueous form as hydrofluoric acid, and thus is the precursor to many important compounds including pharmaceuticals and polymers (e.g. Teflon). HF is widely used in the petrochemical industry and is a component of many superacids. In this study, we quantified, analysed and mapped the HF flows in the chemical industry of South Korea by applying substance flow analysis (SFA). Our result shows that the amount of consumption in each company (and industry), location of manufactures using HF as well as HF's flow (import, export, stocks and flows) in Korean industrial system (between industries including companies). Finally the result of this study can support for chemical accident prevention system and sustainable chemical management in South Korea.

TH109

A Study of Ammonia Substance Flow Analysis in South Korea

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Currently many chemical regulations such as EU REACH and Korea REACH are effected to chemical industry directly. With this regulation, it is required more assessment tools and methods for sustainable management in as well as accidents related with chemicals. Although there has been many researches on how to prevent chemical accidents and manage chemicals, very little attention has been paid on chemical substance flow in country level. For this study, ammonia chemical substance was selected. Ammonia or azane is a compound of nitrogen and hydrogen with the formula NH_3 (CAS number 7664-41-7). It is a colourless gas with a characteristic pungent smell. Ammonia contributes significantly to the nutritional needs of terrestrial organisms by serving as a precursor to food and fertilizers. About 80% of the global ammonia production is consumed by the fertilizer industry; specifically, 48% of the ammonia produced is deployed in the production of urea (the most commonly used nitrogen fertiliser and basic feedstock for industrial products like plastics, resins and adhesive) (Potashcorp, 2013). By using material flow analysis method, ammonia substance flows in South Korea was shown in this study. Required data such as import, export, production and consumption was collected from industry, association and national statistic department. Also spatial location information of manufacturing companies was analysed. Finally this study can support for the chemical accident and risk prevention system and sustainable chemical management in South Korea.

TH110

The DemO3AC Project - Ecotoxikological investigations of an ozonation treatment in the waste water treatment plant Aachen Soers (Germany)

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The European Water Framework Directive aimed to achieve a good ecological and chemical status in all European surface waters until now. However, only 20 % of German surface waters achieved these goals. One reason for this could be anthropogenic pollutants coming from wastewater treatment plants (WWTP).

These pollutants were not completely eliminated in the treatment process and thus enter the aquatic environment. Hence, in the DemO$_3$AC-Project a case study is conducted to demonstrate the implementation of a further treatment step, an ozonation process, to eliminate these pollutants in the WWTP. In this project different ecotoxicological bioassasy were applied to evalute and optimize them in focus for the application in water quality assessment of the river Wurm and the effluent of the WWTP. The river Wurm carries about 70 % of the treated wastewater at low and medium water levels. First, the *Status quo* of the water will be determined and then compared with the data after the implementation of the large-scale ozonation facility. With the standardized test methods for effluent assessment in accordance with DIN and ISO (bacteria, algae, daphnia and fish embryotoxicity) acute and chronic effects of anthropogenic trace substances at different trophic levels will be examined. In addition, cell-toxicological test systems will be used to investigate the estrogenic activity, effects on steroidogenesis and the mutagenic effects of the river water and effluent of the WWTP. On the organisms level *in vivo* and *in situ* studies are planned with the rainbow trout (*Oncorhynchus mykiss*), the mud snail (*Potamopyrgus antipodarum*) and the freshwater shrimp (*Gammarus spp*.). After evaluation the sensitive and most informative tests will be selected. These selected bioassays will be applied for the second stage of the project after the implantation of the new treatment step in the WWTP. A comprehensive chemical analysis of trace substances will be conducted by the Institute of Environmental Engineering (RWTH Aachen, Germany), at the same time-points. In addition, studies on the microbiological composition and antibiotic resistance will be conducted by the Institute of Applied Microbiology (RWTH Aachen, Germany). The project is funded by the Ministry for Climate Protection, Environment, Agriculture, Conservation and Consumer Protection of North Rhine-Westphalia.

TH111

Agrochemical loading in drain and rivers with connection with coastal lagoons in North of Sinaloa, Mexico: a potential risk linking to harmful algal bloom

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Ocean algal blooms have been related to nutrient accumulation in coastal lagoons in the Pacific Ocean. It is speculated that this nutrient overflow has its origins from the agricultural lands in northern Mexico. Sinaloa has a planted area of a million and a half hectares representing 38% of the vegetables produced in the country and ranking first in the production of grains it also excels in the export of sesame and corn. The value of agricultural production in the state varies in the range of 2 million euros a year and participates with six percent of the national agricultural GDP. Currently, annual pesticide application has been calculated to be 700 tons of 17 different types of pesticides which are classified from moderately to strongly toxic according to WHO. This intensive use of agrochemicals has led to the contamination of water bodies and sediments in the area. Therefore, this study aimed to determine the nutrient load and more persistent chemicals (organochlorine pesticides), chemicals that are subject to the Stockholm Convention. These compounds were determined in surface water bodies of Culiacan Valley, particularly in rivers and drains, as well as in the coastal lagoons that the studied rivers and drains fall into. According to the results, except for the Sinaloa and Culiacán rivers, organochlorine pesticides were detected in all samples, from which the only pesticide absent was heptachlor. According to the Rights Act recommended maximum concentrations for the protection of aquatic life in freshwater, brackish or coastal water ecosystems, all sampling sites where pesticides were detected in the water column, exceeded the maximum water quality guidelines for the protection of aquatic life. In most of the studied sites, concentrations of ammonium nitrogen as ammonia, nitrate, nitrite and phosphate, significantly exceeded the maximum allowable limits for the protection of aquatic life guidelines established in the Law on Water Rights, for both freshwater and coastal. These results could be related to harmful algal bloom in the area.

TH112

Phospholipid fatty acid profiles as indicators of microbial community composition variations in soil by mixed heavy metal pollution

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Primary information on heavily contaminated urban areas comes from many cities, however, chemical pollution assays are unable to evaluate the integrated biological effect, which shows a 'cocktail' of environmental factors acting simultaneously on biological systems. This study focuses at assessing of the microbial community structure of urban soils by phospholipid fatty acid analysis (PLFA). The soil samples(topsoil layer 0-20 cm depth) were taken at six sites located in the rural part of the cities of Kirov (Russia), differently polluted with heavy metals.The samples were measured for pH and the contents of organic C, total N, total P and total Pb, Ni, Cr and Cd. The structure of soil microbial communities was assessed using PLFA analysis (high-performance liquid chromatography–mass spectrometry method).The total PLFA content in control sample were found to be significantly higher than the polluted soil. The greatest indication value was given to an *Actinobacteria phylum,* their concentration decrease remarkably in the polluted samples, and anaerobes *Butyrivibrio* sp. and *Bifidobacterium* sp.were found as indicator of soils under relatively low soil pollution status.The microbial profileswere also indicated a selective enrichment of competent species (*Desulfovibrio* sp., *Bacteroides fragilis, Chlamydia* sp., *C. trachomatis*) in soil with high heavy metals contamination. Nonmetric multidimensional scaling plots of soil communities, showing the relative differences in control and pollution soils.Overall, our studycould promote to better understand how microbial community structure might adapt to heavy-metals stress. This study is supported by RSF (14-50-00029).

Multiple stresses in aquatic ecosystems: Assessment of stress response and its consequences in organisms (P)

TH113

Current and emerging issues in bivalves ecotoxicology

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The lifestyle of bivalve populations makes them species at risk to anthropogenic stressors such as pollution, loss of habitats and climate changes. Indeed, mussels are sessile organisms and could live to relatively long periods in some species (up to decades if not centuries). It is anticipated that global changes are likely to have local impacts on biodiversity. In this sense, local mussel populations are directly impacted by upstream disturbances such as urban effluents discharges and loss/modification of habitats. They are filter-feeders from which they trap and concentrate micro-particles in the digestive gland which represent a major vector for contaminant exposure in mussel tissues. For these reasons, bivalves were selected as sentinel species to assess the toxicity of emerging contaminants such as nanotechnology, oil sand products, endocrine disrupters from municipal discharges and changes in the microbiome. The cumulative effects of complex mixtures and other stressors in these times of climate change were also examined at the molecular and cellular levels in the attempt to identify adverse outcomes pathways leading to the decline of mussel populations. Exposure to xenobiotics increase the susceptibility to temperature changes at the electron transport steps in mitochondria which could increase energy expenses in bivalves at polluted sites under thermal fluctuations. Exposure to municipal effluent and zinc oxide nanoparticles both elicit oxidative stress which could lead to inflammation and phagocytosis suppression. Recent studies also showed that air time survival could also be shortened in mussels exposed to nanoparticles and oil sand contaminated environments which support the contention that mussels are species at risk from urban activities. Studies dealing on the cumulative effects of environmental stressors were also performed. Issues: Cumulative effects (nano and ME /Vibrio and ME), Climate change (SOS), endocrine disruption (feminization)/ emerging compound (pharma/OS) and molecular approaches for biodiversity studies (eDNA).

TH114

Evaluation of the health status of the blue mussels Mytilus edulis from two contrasted sites in the Bay of Seine (Normandy, France): A seasonal, transcriptomic survey.

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The blue mussel *Mytilus edulis* is a common bivalve forming large populations on natural and artificial hard substrates. These sessile, filter-feeder organisms are able to bioaccumulate and/or metabolize and tolerate a wide range of pollutants, sometimes at high concentrations. They have been extensively used as bioindicators and sentinel species to assess coastal water quality, especially in estuaries. However, mechanisms by which mussels can survive in such polluted environment are not completely understood. In this context, indigenous mussels were sampled every 3 months during a full annual cycle at two contrasted sites situated on the English Channel coast in Normandy (France): Villerville-sur-Mer (Normandy, France) located at the mouth of Seine estuary, a site highly affected by contaminants originating from agricultural runoff, urban sewage, inputs from industrial plants, and Yport (reference site) located on the north of Le Havre which is not directly exposed to the inputs of the Seine river. Previous studies have demonstrated that a large set of biological endpoints are necessary to detect stress responses generated by a complex contamination in highly variable ecosystems. Hence, a battery of molecular and physiological markers was investigated to allow the characterization of the spatio-temporal variation of the health status of mussels located in the two studied sites. Biomarkers corresponding to energetic metabolism, immunity/inflammation, stress and detoxication were investigated at the transcriptional level (a total of 43 genes) in both gills and digestive gland. In addition, a condition index was calculated and histology was performed in order to assess the health and reproductive status as well as the sex ratio of the mussels. Indeed, in marine bivalves, reproduction is a major biological function especially costly in energy. Results indicated that variations of biomarker responses were associated to seasonal variations, the contaminant levels in the environment and the reproductive status of organisms. These results showed the ability of mussels exposed chronically to contaminations to acclimate to their environment which may contribute to their survival in theBayofSeine. This work was supported by a grant from both ANR and NSERC Agencies (joined France-Canada project) as a part of the research programme IPOC (Interactions between POllution and Climate changes: Development of improved monitoring strategy, ANR-12-ISV7-0004-01, 2013-2015).

TH115

Molecular response of mussels Dreissena sp. to contrasted environments: comparison of different French and Canadian populations

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Zebra mussels from gender *Dreissena* are classically used as model species in ecotoxicological studies assessing freshwater ecosystems quality. These filter feeders sedentary bivalves are considered to faithfully represent their living environment. Numerous biomarkers have been developed in dreissenids as tools of freshwater ecosystems biomonitoring. In the context of IPOC franco-canadian research program (Interactions between POllution and Climate changes: development of improved monitoring strategy), *Dreissena sp*. was retained as freshwater model mussel to evaluate biological effects of combined action of pollution and climate change, following French and Canadian populations health. Populations of mussels from four rivers in the north of France (Vilaine, Moselle, Meuse and Oise) were sampled in spring and autumn. In parallel, Canadian populations of mussels from four different sites along the St Laurent River were also sampled in autumn. Condition index was measured. Contaminants contents were analyzed. Molecular response of the different populations were assessed through the following of gene expression by real-time PCR. Candidate genes were chosen for their involvement in cellular energy metabolism (cytochrome-C-oxidase, ATP synthase), antioxidant systems (catalase, superoxide dismutase, glutathione peroxidase) and xenobiotics detoxification (glutathione-S-transferase, metallothionein). Results showed that French mussels tend to present a higher expression level for most of genes in the site considered as the less contaminated. In this site, seasonal variations were more pronounced than in the three others, with expression levels of candidate genes lower in autumn than spring. First genetic analysis also revealed that some French mussel samples presented two dreissenid species: zebra mussel *Dreissena polymorpha* but also quagga mussel *Dreissena rostriformis bugensis*. This second species appeared as the most widely represented in Canadian populations. As in French populations, expression levels of chosen genes were often higher in mussels sampled in the less anthropized site of the St Laurent. This study shows that mussels from different sites could be discriminated with expression gene responses, what constitutes a promising point to evaluate population health status.

TH116

Physiological state and metal bioaccumulation in mussels, Mytilus edulis, transplanted into a mesohalin marina in Normandy (North West France)

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Mussels are often used as sentinel organisms in biomonitoring surveys performed in coastal environments such as Mussel Watch Program (NOAA; USA) or ROCCH (IFREMER; France). *Mytilus ssp* are well adapted because they are sessile, filter-feeder and euryoecious mollusks. Environmental monitoring surveys generally include one-off sampling in the year and do not take into account the temporal variations in contaminant burden and biological parameters. Moreover, the fluctuation of environmental parameters can constitute confounding factors in the detection of species responses to habitat contamination. In this context, we assessed the monthly variability of the bioaccumulation of trace metals and a battery of biomarkers in mussels caged in a constraining environment. Before the beginning of the experiment, a batch of *Mytilus edulis* originating from Chausey Islands (English Channel, Normandy, France) was progressively acclimated to low salinities (from 32 to 12 PSU). Then, mussels were continuously caged into the marina of Carentan (Normandy) which is predominantly supplied with freshwater from two rivers but also influenced by seawater from the Bay of Veys. Finally, this marina can be considered as a mesohalin station where we placed a high-frequency probe which recorded temperature and salinity. In 2015, samples of water and mussels were monthly collected for metal analyses, and various endpoints at different levels of biological organization were studied. Individual biomarkers included the assessment of mortality and growth rates, and histology allowed to determine the reproduction success and tissue quality (histopathology). The regulation of oxidative stress was evaluated by measuring both enzymes activities (catalase and glutathion-s-transferase) and the final product of lipid peroxidation (malondialdehyde). Neutral red uptake assay gave information about lysosomal system while flow cytometry approach provided data about reactive oxygen species (ROS) and hemocyte phagocytic activity. One month after the beginning of the study, high mortality rates were recorded but then mussels showed a high ability to survive (up to 10 months) in a contaminated environment where salinity greatly varies and where mussels had thus to face multiple stresses. The present study highlighted the temporal fluctuations of some endpoints and these variations should be taken into consideration in the interpretation of data obtained from biomonitoring surveys.

TH117

A step forward in multixenobiotic resistance: first evidence of ABCG2-type gene expression and effect of season change and contaminants on ABC mRNA expression in gills and hemocytes of the blue mussel

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Multixenobiotic resistance (MXR) is a powerful defence system mediated by transmembrane proteins known as ATP binding cassette (ABC) transporters that bind and actively remove xenobiotics from the cell. The aim of this study was i) to identify the gene encoding the breast cancer resistance associated protein (BCRP) in hemocyte and gills of *Mytilus edulis*, ii) to explore the effect of season change and site pollution on the gene expression of three ABC transporters identified in the blue mussel (ABCB, ABCC and ABCG2) and iii) to correlate transcripts levels in gills with efflux transporter activities. The expression of ABCG2/BCRP transporter was investigated in gills and hemocyte extracts by RT PCR and 5'-RACE. The PCR products had the expected size in both tissues. The identified partial cDNA sequence of ABCG2 overlapped with ABCG2 homologues of other organisms from 72 % to 98 %. The expression quantification of abcb-like, abcc-like and abcg2-like genes was carried out using RT-QPCR on mussel's extracts collected during 4 seasons from areas identified by the program IPOC (Interactions between POllution and Climate changes) for being differentially impacted by chemical contaminants in Normandy. Results indicate that transcripts levels of ABC transporters in gills and hemocytes significantly fluctuated along seasons. Overall, we observed an increase in ABC gene expression during February for both tissues. In hemocytes, abcb-like and abcg2-like transcripts were more abundant in mussels originating from the control site during February than those collected from the contaminated site at the same period. The trend was reversed for the other seasons where abcg2-like expression increased significantly in the control area. Furthermore, no relevant change was noticed for abcc-like expression. In contrast, gills exposed to pollutants showed a higher abcb-like and abcg2-like transcript levels in February and conversely a lower abcg2-like expression while no difference was noted for the other seasons. We also studied the efflux activity by measuring the accumulation of calcein in gills in presence of ABCB and ABCC blockers. A higher transporter activity was measured in tissues obtained from the contaminated site which is concordant with the transcripts levels. All together, the present results suggest the regulation of ABC transporters by season change and water contaminants besides their involvement as protective and tissue specific response xenobiotics.

TH118

Effects of a realistic multiple low dose pesticide exposure scenario on aquatic communities in stream mesocosms

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The current common EU risk assessment for pesticides refers to effects of single substances. As one result of this risk assessment a regulatory acceptable concentration (RAC) for each pesticide is estimated, which should not lead to any inacceptable adverse effect on non-target organisms in freshwater systems. However, in agriculture combinations of different pesticides are repeatedly used over time and space to protect crop from pests. Hence, communities inhabiting streams in agricultural landscapes are exposed to mixtures of pesticides and their metabolites, which might cause a multiple-stress scenario for these non-target organisms. For example, in orchards a high number of pesticides with different modes of action are repeatedly applied in short time intervals during the whole vegetation period. However, potential additive or even synergistic effects of realistic exposure scenarios are rarely investigated, especially on the community level. Here, we studied the effects of a realistic pesticide spray scenario for apple crop protection on aquatic communities in surface waters using eight indoor stream mesocosms. Aquatic invertebrates from a reference site were added to the stream mesocosms using colonized straw bags. Overall, nine fungicides, four insecticides and four herbicides were applied at their respective RACs for surface waters to each of the four treatment mesocosms within a period of two and a half months. At nineteen days altogether pesticides were used 36 times with up to four different pesticides per date. Most fungicides were used repeatedly up to seven times. In order to detect potential effects on the aquatic community we measured a variety of biological endpoints. We focused mainly on sublethal effects, which are more likely to occur in a multiple low dose pesticide exposure scenario. Potential short-term effects like behavioral changes within several hours were determined by measuring drift and activity patterns of benthic invertebrates. Long-term effects were measured by changes in the community composition of invertebrates and the emergence of merolimnic insects. Furthermore, we investigated potential effects within the food web structure using stable isotope analysis and fitness of invertebrates using the RNA:DNA ratio as proxy. We compared the results of different endpoints in order to quantify the impact of the multiple stress scenarios on different scales.

TH119

Identification of relevant toxicant mixtures influencing the macroinvertebrate community of the Danube river

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Aquatic organisms inhabiting large rivers are exposed to a wide range of contaminants including down-the-drain chemicals (e.g. pharmaceuticals, food additives, home-care products), industrial pollutants (e.g. metals, PAHs) and pesticides. It is therefore evident that observed changes in biodiversity are likely to be explained by toxicant mixtures resulting in additive, synergistic or antagonistic responses at the individual, population and community levels. Besides the large number compounds and possible mixtures occurring in the environment, it is expected that only a limited number of substances are responsible for the observed biological responses. Identifying these chemical mixtures is key to understand their combined toxicity mechanisms and to derive responsible risk abatement options. The main objective of the current study was to identify toxicant mixtures that are influencing the macroinvertebrate community monitored in the Danube river. A large database obtained as part of the environmental monitoring campaign performed during 2013 in the Danube river was used in this study. The database contained abundance data for 393 invertebrate species monitored in 55 sampling sites of the Danube river, and measured chemical concentrations in each sampling site. The chemical dataset contained water concentrations for 227 chemicals, including pharmaceuticals, pesticides, PAHs, metals, food additives, and home-care products. Prior to the mixture toxicity identification, four biological indices were calculated that represent the macroinvertebrate community in each of the sampling sites. These were: taxa richness, total abundance of individuals, Shannon diversity index and species evenness. Chemical mixtures significantly influencing the variability in the community indices were identified by selecting best-fit Generalized Linear Models (GLMs) containing binary, tertiary, quaternary and quinary chemical combinations. The selection of best fitting models was performed using the RSELECT procedure in the GENSTAT software. The best fitting models were obtained for species richness, with R^2 values of up to 63%. Relevant chemical mixtures included, in most of the cases, pharmaceuticals (e.g. anti-depressive and anaesthetic drugs), insecticides, and some feed additives. Further research should aim to study the mechanistic link between the identified chemical mixtures and the community responses using laboratory and/or semi-field experiments.

TH120

Characterizing a nanocosm for the evaluation of toxicant effects on an aquatic community

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When organisms are exposed to toxicants they can be influenced directly when e.g. feeding or reproduction are impaired and indirectly when interactions with other system components are altered. These ecological effects can be studied in micro-/and mesocosms but they are expensive, time and labour demanding and, in general, few replicates are used. With ecological complexity the variability between replicates increases and an extrapolation of effect/response relationships is more difficult. In regulatory risk assessment, a tiered effect assessment approach is in use to evaluate the effects of pesticides on non-target organisms but only when the direct effect results of single-species tests (inexpensive, quick and repeatable) raise concern, are micro- or mesocoms employed. Few small systems have been developed to describe direct and indirect toxicant impacts on multi-trophic communities and they are rarely tested for reproducibility. This project aims to develop and characterize a small, multi-trophic aquatic test system to bridge simple single-species tests and complex multi-factor micro-/mesocosms. Species with short life cycles and fast reproduction times are used to keep test durations short and reproducibility is increased by using a simple test design. This allows the assessment of toxicant impacts on basic community processes and system dynamics, and the extrapolation of causal/mechanisms. The system will be challenged with pesticides and the outcome will be compared to the predictions made by an Individual Based Model (IBM) developed with data obtained in single-species tests. It will be evaluated whether effect data of system components are sufficient to predict community dynamics or if it is necessary to assess the impacts on the system as a whole (including species interactions). Here we present on overview of the nanocosm development, experimental protocols and control variability.

TH121

Oscillating population dynamics: chemostats to study direct and indirect toxic responses in simple prey-predator system

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Keywords: Chemostats, *Daphnia magna*, algae, atrazine, Understanding the impacts of natural and anthropogenic stressors on ecosystems requires analyzing responses at the level of ecological interactions between individuals and populations. This obviously applies also to the analysis of the impacts of micropullutants in the environment. Traditionally, ecotoxicology opted for a highly reductionist frame focusing on responses of individual populations treated in static environments. This approach totally neglects the cascade of effects that chemical stressors can produce by influencing ecological interactions across competing groups or along a trophic chain. Excess of reductionism can therefore lead to misinterpreting the real scale of contaminant impacts in the ecosystem, while tolls for investigating propagation of effects at higher ecological levels are still not well established. We developed a chemostat-based tool which allow reproducing "simple", self-regulating and ecologically dynamic systems (e.g. primary producer-grazed system). Through the chemostats we conducted experiments to investigate chemical (or other stressors) impacts in a simplified, though fully functional, biological system. Chemostats with oscillating cycles of a primary producer population (*Pseudokirchneriella subcapitata*) and a grazer population (*Daphnia magna*) where obtained and maintained over 3 months. A set of chemostats were routinely spiked with Atrazine to maintain this photosynthesis inhibitor close to the $EC50_{algae}$ and below $NOEC_{Daphnia}$. The addition of the atrazine to the dynamic system modified trophic transfer affecting Daphnia population dynamics despite atrazine, at these exposure levels, did not have any direct effect on the grazer population. The Chemostat was therefore successfully used to illustrate how impact of chemicals can be transferred across trophic levels and produce implication for the biological system functioning as a whole.

TH122

The effect of selected biocides on algae communities in water bodies affected by stormwater runoff

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In urbanished areas many biocides are in use to prevent unwanted growth of organisms on e.g. construction materials and wood. Over time, the biocides leach from the urban materials and are transported with stormwater runoff to receiving water bodies. As biocides are non-selective, they can affect non-target organisms and thereby cause unintended harm to the surrounding environment. A common approach for stormwater management is to establish wet detention ponds. They fulfill dual purpuses. Firstly, they detain the runoff water during heavy rain and hereby reduce the hydraulic loads on the receiving waters. Secondly, they hold stormwater runoff for prolonged durations, allowing natural treatment processes to proceed. Due to their permanent water pool they furthermore present themselves as habitats for flora and fauna, and rapidly become populated with species similar to what is found in natural ponds. The biota in the stormwater ponds hence become subjected to the biocides from the building materials. This study focusses on four biocides; terbutryn, diuron, irgarol and carbendazim, and their effect on the algae growth and change in the community composition at conditions found in stormwater ponds. The biocides were studied in three test systems. Two were microcosms at controlled laboratory conditions with stabile temperature and a light exposure of 12 h per day. These ran for 10 days, with algae sampling at day 1, 5 and 10. The first setup was conducted in glass bottles filled with pond water. Water samples, from a wet detention pond in the city of Silkeborg, was collected from 5 locations allocate evenly in the pond and mixed thoroughly. The second controlled microcosms study was performed in glass sediment cores, filled with pond sediments and water from the same pond. For both setups, stock solutions of all biocides were added in varied concentrations. The third setup consisted of mesocosms placed in the pond from where also the water and sediments had been sampled. Here, the added biocides and algae community were exposed to natural changes in temperature, sunlight and rain. This experiment ran for 15 days, with algae and biocide sampling several times during the period of exposure. The development in algae communities and its dependence on biocide levels were compared in the 3 setups, yielding results on the impact of the biocides on community composition and on cell numbers and cell sizes.

TH123

ECOSCOPE: a mesocosm facility to evaluate stressor impacts on headwater streams across multiple levels of biological organization

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Predicting long term consequences of stressors is a key challenge of environmental risk assessment. Most of the developed methodologies rely on either standardized toxicity tests (for *a priori* evaluation) or on well known bioevaluation methods (for *a posteriori* evaluation). However, long term effects of stressors on aquatic ecosystems are still poorly explored. Besides that, usual protocols generally focus on only one compartment and/or biological scale, neglecting biotic interactions, functional processes and more typically effect propagation, top-down feedback and ecological non-monotonic responses. To help the scientific community to address these issues, an outdoor mesocosm platform with 18 artificial channels of 3 meter long, with water circulation and roof system has been built to simulate headwater streams, and to explore the responses of the ecosystem set in place on the long term (several months), with a systemic approach taking into account several biological compartments (macroinvertebrates, bacteria, fungi, algae...). The channels are designed to allow for changes in flow velocity, thermal and chemical conditions and habitat structure, and, obviously to combine those abiotic parameters with contaminants to explore multi-stress conditions. They are equipped with sensors for a real-time tracking of temperature, oxygen concentration and pH. The platform is open for research project in collaboration with academia, stakeholders and private investigators.

TH124

A fish multi-biomarker approach, including main physiological functions, to better determined global effect of contaminant on ecosystem

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Risk evaluation, due to the presence of toxic compounds in the environment, brings up the necessity to better understand their global effect on ecosystem. In this way, in addition to traditional biochemical biomarkers, some laboratories proposed to study the responses of biomarkers in relationship with the main physiological functions (reproduction, survival, maintenance, behaviour). This approach favors the evaluation of links between biochemical responses and individual/population effects. In this context, a multi-biomarker approach was used in four stations of a French river (La Loue – Franche-Comté region) surrounding area of drastic fish mortalities. In each station, 20 bullheads of both sexes were caught in spring and autumn by electrofishing for further biomarker analyses. The major results shown that the global health indicator (index condition, lysosomal membrane integrity and lipoperoxydation), the indicator of reproduction (gonado-somatic index and the maturation stage), the biotransformation enzymes and chromosomique damages were never modified regardless to station. Nevertheless, immune response and neurotoxic activity were strongly modified in the stations with fish decline. All these results could imply an important immune request and/or the presence of neurotoxic compounds in the river, inducing fish mortalities. This study showed the relevance of using multi-biomarker approach, including main physiological functions, to better determined effects of various contaminants on ecosystem health.

TH125

The three spined-stickleback's digestive enzymes as new biomarkers in environmental monitoring: effect of cadmium and temperature

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The three-spined stickleback, is a freshwater, brackish, and marine fish species with large distribution throughout the northern Hemisphere. This pollution-tolerant fish is present in most European streams and small rivers but also coastal and estuarian areas, which make it a suitable candidate for environmental monitoring and risk assessment of aquatic ecosystems. Several biomarkers have been developed for this animal model. This study aims at characterizing and developing a new category of biomarkers in relation with energy acquisition, in sticklebacks. Among these parameters, digestive capacities of many invertebrates and vertebrates aquatic organisms have demonstrated a good sensitivity to contaminants. Thus, in this context, the most important digestive enzymes were firstly characterized in this sentinel species, according to it diet, in optimal living conditions. Secondly male and female juvenile sticklebacks were exposed for 3 months in semi-static conditions to cadmium at $1\mu g.L^{-1}$ (chemical stress), and two temperature values 16°C and 21°C (physical stress). Water quality and cadmium concentration in the water were also monitored throughout the experiment. Parameters such as somatic indexes (height, weight, fulton's condition index) and digestive enzymes (trypsin, intestinal alkaline phosphatase and amylase) were measured at 15, 60 and 90 days interval after cadmium exposure. The results have demonstrated a significant decrease in biometric parameters, from the 3rd month, especially among groups subjected to a temperature of 21 ° C. Furthermore, we observed an important alteration of the activity of the different enzymes over time and especially for trypsin and alkaline phosphatase in individuals exposed to cadmium at 21°C suggesting interactions between chemical and physical stresses on biological responses. For the first time, the digestive enzymes of the three-spined stickleback are reported as new environmental biomarkers.

TH126

Pesticide effects on stream fungi in a realistic apple-crop exposure scenario

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Significant amounts of pesticides applied in agriculture may reach surface waters via spray drift or runoff. However, the impact of pesticides on aquatic fungi has rarely been investigated, although fungi in streams and other inland waters play a key role in the decomposition of organic matter such as plant litter. Here we assessed the effects of pesticides on aquatic fungi in a stream mesocosm experiment conducted in a large indoor stream facility. The experiment was designed to mimic a realistic apple-crop exposure scenario involving multiple low-dose pulses of pesticides. Our specific goals were to assess whether (i) quantitative polymerase chain reaction (qPCR) is a useful method to monitor the aquatic fungi abundance; (ii) aquatic fungi are affected by pesticide pulses at low doses; and (iii) individual fungal species can serve as indicators of pesticide impacts. Dried alder leaves (*Alnus glutinosa*) were inoculated with suspensions of homogenized mycelium obtained from two strains of aquatic hyphomycetes, *Tetracladium marchalianum* and *Neonectria lugdunensis,* and one strain of the ubiquitous fungus *Cladosporium herbarum* and exposed in eight stream mesocosms. Four mesocosms served as untreated controls. The other four were dosed seven times with six different fungicides, once with two herbicides and twice with two insecticides. 'Regulatory Acceptable Concentrations' (RAC) of these pesticides were applied on a total of eight days within two months. Up to four different fungicides were applied on a single day. Endpoints included abundance (qPCR) of the three target fungi, ergosterol as an indicator of total fungal biomass, and fungal spore production. Rates on leaf litter breakdown were also determined. Results are discussed in relation to their implications for risk assessment of pesticides.

TH127

Combined toxicity effects of binary pesticide mixtures on luminescence bacteria Vibrio fischeri

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Pesticide have been mostly used as plant protection products (PPP) for agricultural and non-agricultural purposes. Various pesticides released to the aquatic environment have been found in the form of mixtures. In this study, luminescence marine bacteria *Vibrio fischeri* was used as a target living organism to measure toxicities of 7 single pesticides (chlorpropham, dimethoate, diquat dibromide monohydrate, acetamiprid, clomazone, dazomet, and dodine) which were selected in a list of PPP and their 21 binary mixtures. Additionally, to classify the combined effects at equitoxic ratio, toxic units (TU) was calculated with concentration of single chemical in mixture divided by EC_{50} value of their single chemicals. Theoretically, the concept of 1TU is assumed as additivity, but the combined effects were classified as additivity when TU ranges from 0.8 to 1.2 considering human errors in this result. Based on TU values, 10% of total mixtures showed synergistic effect and 38% and 52% of mixtures indicated additive and antagonistic effects at EC_{50} level, respectively. As single chemicals, dimethoate, chlorpropham, dazomet, clomazone and acetamiprid seem to be closely related to synergistic effect, while the most number of dodine and diquat were found in antagonistic combinations, suggesting that dodine and diquat are strongly involved in antagonistic mechanisms. Interestingly, dodine was much more involved in combinations having antagonistic effects, though it had considerably higher toxicity than six pesticides at EC_{50} level.

TH128

Preliminary approach for the study of the effects of multiple stress factors on honeybees

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Bee decline is a problem of high concern all over the world. Since the late '90s, a complex pathology (Colony Collapse Disorder: CCD) brought to widespread events of honeybee disappearance especially in the U.S. and in Europe. It was not always possible to relate them to a specific cause. Many adversities may be responsible of CCD events and of the general honeybee decline: recrudescence of old and new pathologies, environmental stresses, including climate change, contamination from pesticides and emerging contaminants (e.g. nanoparticles) and electromagnetic fields. All these factors may interact among them and show synergic effects or multiple exposure ways. This work is a preliminary attempt to evaluate the effect of combined stress factors (pesticide mixtures and electromagnetic fields) on honeybees. Three experimental sites where selected in northern Italy: a control site, far from agricultural field and from any significant human settlements; an agricultural site, located in a farm where different crops (orchards) are present with a complex and controlled situation of pesticide application; a semi-natural site, far from agricultural areas and close to an electric line generating a strong electromagnetic field. In each site some experimental hives were posed. The sites were sampled monthly from May to October 2015. At each sampling date a suitable number of bee (both workers and standing) were sampled for biochemical (acetylcholinesterase, lipid peroxidation, antioxidant enzymes) and genetic (Comet assay) biomarkers. Some observation were made on the health status of the colony. Moreover, electromagnetic field measurements were performed and samples of environmental matrices were collected for quantifying pesticide exposure and for calibrating suitable exposure models. Meteorological parameters were get from the meteorological station closer to each sampling site. The effects of different stress factors are evaluated and compared. The work will be used as pilot project for the development of a more extensive research project on multi-stress effects on bee decline.

TH129
The sublethal effects of epoxiconazole and α-cypermethrin toward Daphnia magna reproduction and neonates' size
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Azole fungicides and pyrethroid insecticides, two classes of pesticides commonly used in agriculture, can reach surface water due to spray drift and run off after rain events. Because of the opportunity of both azoles and pyrethroids to enter surface water, and since azoles have been shown to synergize the effect of pyrethroids, the effect of their mixture is of concern. In particular, the sublethal effects of low concentrations are of interest, since these are the concentrations most often occurring in the environment. Beside the commonly described adverse effects of pesticide exposure on mortality, growth and reproduction, it is also generally recognized, that exposure to low doses of insecticides may result in hormetic responses. Hormesis is defined as a biphasic response, where the effect of exposure to low doses is opposite than the effect at high doses. One of the proposed explanations for hormesis due to low concentrations of insecticides is an initiation of the induction of detoxification enzymes such as cytochrome P450. The aim of the present study was to investigate the effect of sublethal doses of an azole fungicide (epoxiconazole) and a pyrethroid insecticide (α-cypermethrin) and their mixture on growth, reproduction and *in vivo* cytochrome P450 activity of the aquatic crustacean *D. manga*. The study was designed to investigate the following hypotheses: 1) azole fungicides act as synergists of pyrethroid insecticides also at sublethal endpoints such as growth and reproduction 2) a possible trade-off between size and number of neonates may be present for the organisms exposed either to single compounds or to the mixture and 3) low doses of epoxiconazole stimulate cytochrome P450 activity *in vivo* thereby a) increasing the detoxification of α-cypermethrin resulting in less harm to reproduction or b) increasing metabolic costs of detoxification leading to lower growth and/or reproduction. The experiment was designed to separate and count neonates over time, while measuring length and protein content of mothers and neonates as well as cytochrome P450 activity of mothers over time. The cumulative reproduction of *D. magna* varied according to time and exposure. The reproduction observed in the different treatments will be compared and associated with length and protein content of both mothers and neonates and with cytochrome P450 activity of the mothers in order to address the three hypotheses.

TH130
Physiological state and metal bioaccumulation in the alien mussel, Mytilopsis leucophaeata, sampled in a freshwater site and a mesohalin marina in Normandy (North West France).
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The brackish water mussel, *Mytilopsis leucophaeata* is a species from the Dreissenidae family originated from the south of U.S. Atlantic coast (Mexico Gulf to Hudson River Estuary). Nowadays, this species spreads in Europe from Spain to Scandinavian countries, and is considered as a potential invasive species. By comparison to other mussel species such as *Dreissena polymorpha,* few studies have been conducted on *M. leucophaeata,* and most of them were performed on antifouling solutions and its new distribution areas while very few studies dealt with ecotoxicology. However, *M. leucophaeata* should be well adapted to biomonitoring surveys as this species is sessile, filter-feeder and euryoecious. Moreover, this species can live in a wide range of habitats from freshwater to mesohalin areas giving the possibility of environmental monitoring along the land-sea continuum. The aim of this one-year study was to evaluate the adaptation mechanisms developed by *M. leucophaeata* in two contrasted areas (a marina and a river) through the monthly monitoring of metallic contamination and a battery of biomarkers at different levels of biological organization. The battery of selected biomarkers included endpoints from individual scale (survival, biometric parameters) down to infra-individual descriptors, such as histological (determination of gender and gametogenesis stages) and biochemical biomarkers (activities of glutathione-S-transferases (GST) and catalases (CAT), and malonedialdehyde as a marker of lipid peroxidation).The results currently available show a greater contamination by trace metals in mussels from the marina with higher concentrations in Cu (up to 10-fold) and Cd (up to 3-fold). Moreover, 4 months after the beginning of the study (mid-April), mussel mortality rates are high, especially in the marina where they reach ~98% *versus* 41% in the freshwater site. At all sampling dates, the mussels from the marina are globally smaller and lighter, and show a poorer condition index. Regulation of oxidative stress does not reveal significant differences between the individuals from the two sites regarding GST activities whereas CAT activities are enhanced at the freshwater site. In order to reveal more inter-site differences, results will be completed by data provided by other sampling dates and other endpoints. Finally, these results are encouraging for the use of *M. leucophaeata*in ecotoxicology and biomonitoring surveys.

TH131
Effects of a combined chemical and biological stresses on fish immune system.
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The ecological and chemical deterioration of rivers water quality is a fact. Several studies highlight that a large variety of compounds detected in French rivers could impact physiology of aquatic organisms. The immune system, in particular innate immune response, seems to be very attractive for biomonitoring due to their connection with organism health status. In fact, this innate immune response is a part of the first line defense in the immune system of organisms acting against pathogens without prior exposure to any particular microorganism. Moreover, organism's defenses may be modified by pollutants leading to increase effect of endotoxin. In this context, the stresses induce by chemical (chlorpyrifos) and combined chemical/pathogens (LPS endotoxin) were determined in the three-spined stickleback, *Gasterosteus aculeatus*, after 4 days of exposure. For chlorpyrifos, dissimilar effects were obtained when fish were exposed to chemical stress alone or combined chemical/endotoxin stresses. An increase of the oxidative burst was observed in fish exposed at high doses of chlorpyrifos. Moreover, the injection of LPS increases immune response at low concentration of chlorpyrifos whereas at higher concentration of chlorpyrifos the immune response was lower than control values. Lysosomal membrane integrity was disrupted by the double contamination, at low and high doses of chlorpyrifos. This study shown that chlorpyrifos have hazardous effects on susceptibility of fish immune system to pathogens.

TH132
The effects of nutrient and pesticide mixtures on the Australian midge Chironomus tepperi
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Phosphate is one of the most common forms of nutrient contamination globally and can cause problems when found in high concentrations. It often occurs in mixtures with many different kinds of toxicants, one of the most common being pesticides. Little is known about the impacts of nutrients on the toxicity of pesticides, despite their common co-occurrence. The aim of this study is to identify the impact increasing concentrations of phosphate have on the survival, growth, and protein concentration of the Australian midge *Chironomus tepperi* and whether these levels of phosphate alter their ability to cope with increasing concentrations of the synthetic pyrethroid insecticide permethrin. Sub-lethal concentrations of both phosphate and permethrin were used in a 96-hr sediment exposure on 2nd instar *C. tepperi* larvae and the survival, growth, and protein concentration of the larvae were measured. In two experiments fifteen 2nd-instar larvae were exposed to spiked sediment and water over 96-hrs. For the phosphate exposure, phosphate concentrations of 0, Control (0.5), 10, and 40 mg/L were created and used as the overlying water for clean sediment. For the permethrin/phosphate exposure, normal and high solutions were created with 0.5

(Control) and 40 mg/L of phosphate and used as the overlying water for sediment spiked with permethrin at low, medium, and high concentrations (62, 250, and 2000 μg/kg). The results showed that the phosphate addition increased the protein concentration in the larvae at higher concentrations (p=0.49), but had no effect on survival or growth. In the permethrin/phosphate exposure high concentrations of permethrin reduced survival regardless of phosphate addition (p=.016). However, when exposed to low concentrations of permethrin the addition of high concentrations of phosphate significantly increased the protein concentration found in *C. tepperi* larvae, but did not affect their growth (p=.001). Our results indicate that increased levels of phosphate may protect organisms from the adverse effects of low concentrations of other pollutants.

Identification and prioritisation of hazardous pollutants in the aquatic environment - the role of effect-directed analysis, monitoring and modelling (P)

TH133

Disentangling ecosystem stressors along a river continuum covering a pollution gradient

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Environmental pollution is intrinsically linked to the way humans live. Many of the environmental problems we face today have existed for decades; what has changed is our understanding of the key drivers, processes and impacts. In 2009, the reporting by Member States (MS) across the European Union (in 6-yr cycles) on the status of their water bodies found that rivers and transitional waters were often in worse condition than lakes and coastal waters. This is not surprising considering that streams integrate all of the diverse stressors found within a catchment (e.g. contaminated sites; diffuse source pollution; water abstraction). The chemical status of a water body is relatively straightforward to assess, defined in part by environmental quality standards on priority substances and in part by additional regulations imposed by individual MS. However, the biological quality elements used for the classification of ecological status are only loosely defined, leaving MS free to develop their own assessment tools. Although useful for the individual MS, it impedes methodological standardization across different ecoregions, thus contributing to inconsistencies and data gaps across Europe. Moreover, despite the unambiguous importance of benthic habitats for overall ecosystem health, many biological indices tend only to reflect the ecological quality of surface water, rather than of the sedimentary zones where the accumulation of pollutants is often highest. To address this issue, we monitored meiobenthic (i.e. nematodes) and macrobenthic invertebrates along a pollution gradient in order to assess the impact of multiple stressors on a groundwater-fed stream, and thus, to quantify the link between chemical and ecological status. Physical conditions were comparable among sites. The studied stressors included point source pollutants originating from contaminated groundwater and aquaculture, and diffuse source pollutants originating from conventional agriculture and urban areas. The results indicate a change in community composition for both meio- and macrobenthic fauna, pointing towards the presence of a local impact resulting from the discharging contaminated groundwater, which extends downstream along a dilution gradient of the groundwater contaminants. Ecological impacts could be linked to xenobiotic compounds coming from groundwater (both chlorinated solvents and pharmaceuticals), as well as the presence of trace metals (particularly copper and aluminum).

TH134

Surfactants homologues profiling by non-target screening in sediment and Cladophora extract from freshwater river.

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Due to the wide use of surfactants, in personal care product, pharmaceutical, pesticides, their presence is rather ubiquitous in the environment. Previous studies have shown that surfactants may be found in the aquatic environment at concentrations, which may be orders of magnitude above other micro-pollutants and result in notably high hazard quotients. Due to their amphiphilic properties surfactants are likely to sorb on surface. Previous work has shown their presence in sediment, but few have been investigating *Cladophora* as potential accumulation compartment. *Cladophora* are often found in nutrient rich river waters downstream of WWTP output, a known emission source of surfactant in the environment. The monitoring of chemicals like surfactants, in these algae will help to estimate the exposure and risks of the respective aquatic ecosystem. In addition, the traditional approach to focus on a few target surfactants for environmental monitoring could underestimate the presence of surfactants in aquatic ecosystems. Indeed, surfactants are often produced together with other homologues having different chain length, these surfactant profiles can as well be found in the environments. Therefore non-target screening of surfactant homologues would bring a better understanding of the fate of surfactant in aquatic ecosystem. In a case study, Holtemme River in Saxony-Anhalt (Germany), which is impacted by WWTP effluents, has been sampled for sediment and *Cladophora*. Non-target screening with a special focus on surfactant homologues was performed on in several compartments, such as sediment and *Cladophora*. The algal toxicity of the environmental fraction was performed too. The method used for this study includes sequential extraction by Pressurized Liquid Extraction, combined with further clean-up steps; the chemical analysis was performed by LC-ESI-LTQ-Orbitrap-MS analysis. Longitudinal profiles of surfactants homologues in the Holtemme River and several of it's tributaries have been identified. Point sources of surfactants have been confirmed hot spots for subsequent in-depth investigations have been identified.

TH135

A non-target screening method for contaminants in fish

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Compared with target analysis, non-target screening provides more comprehensive information on the chemical burden of fish tissues involving potentially pervasive but unknown compounds. In this study, a non-target screening method was developed to detected contaminants in fish muscle. Tissues are lyophilized and then extracted, purified by QuEChERS and subjected to non-target screening in both negative and positive mode with UPLC-Orbitrap-MS. Method development was based on 105 compounds covering a wide range of physiochemical properties and including chemicals that have been found in fish tissue before. The recoveries ranged from 6-167% for all the compounds and 70-110% for most of the compounds. The matrix effects were also in acceptable levels, mostly varying from 80-120%. The method was applied to assess the chemical exposure of contaminants in the fish collected from River Holtemme, Germany. Several contaminants were identified in the fish including naphthalene, benzophenone, fluoranthene, cypermethrin, 4-octylphenol, dibenzylether, carbezole, tonalide and triclosan-methyl. Further quantitative analysis revealed that cypermethrin and 4-octylphenol were the predominant contaminants in the ng/g dry weight range. Moreover, the accumulation patterns of contaminants in the fish identified by the approach were well in accordance with the input of known contamination sources. The preliminary application indicates that the non-target screening method is a promising tool to disclose the chemical exposure and identify the predominant contaminant/s in fish.

TH136

Biomarker response analysis in fish within the SOLUTIONS project - Case study Holtemme

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Close collaboration with several case studies is one of the main advantages in terms of the realization of biomarker response analysis within the 7th EU RTD Framework Programme project SOLUTIONS (Solutions for present and emerging pollutants in land and water resource management). By using the infrastructure of these studies, biomarker monitoring approaches can be performed easily. Under the lead of the Helmholtz-Centre for Environmental Research the small river Holtemme was investigated in an interdisciplinary approach. The stream has its source in the Mountain Harz region (Germany) and flows through a number of small and medium-sized cities in Saxony-Anhalt. Due to its low water-quantity and several anthropogenic point sources the Holtemme is a very valuable model site to assess the impact of pollution induced biomarker responses in aquatic organisms. 10 individuals of brown trout (*Salmo trutta*) were collected at each of 4 different locations including a “natural” reference site. Locations were downstream waste water treatment plants (WWTPs) and one location in a more agriculturally influenced river section. The utilized biomarker battery covered a broad range of proven endpoints which have been employed in several previous studies: genotoxicity in peripheral erythrocytes (micronucleus test), biotransformation enzyme activity (EROD, GST, CES), oxidative stress (TBARS, CAT) and biomarker of exposure to organophosphate and carbamate pesticides (AChE). To reduce time and sample consumption all common cuvette analysis for these endpoints were adapted to 96-well plate assays. For most of the investigated biomarkers significantly elevated/lowered values for the urban and agriculture-affected sampling sites could be demonstrated in comparison to the reference site. However, the results indicated no similar trend for all biomarker responses along the longitudinal profile of the River Holtemme, but the individual biomarkers gave “hot-spots” of response. Correlation analysis with chemical data might help to identify presumed stressors related to the different observed effects.

TH137

Simultaneous determination of PAH metabolites in fish bile by gas chromatography-tandom mass spectrometry

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Recently, there have been frequent oil-spills along Korean coastal areas. So many studies have been conducted to assess the marine environment contamination and impacts on the ecosystems. Among the numerous oil originated contaminants, most of the studies have focused on parent pollutants. However, after organisms are exposed to pollutants such as PAHs, xenobiotic enzymes are released in response for oxidation, reduction, hydrolysis and other processes leading to the breaking down of the parent compound to metabolites. The focus of most pollution assessment is often on the parent pollutant not the metabolites though the parent compound has been partially or completely transformed. Therefore, in this study, we aimed to develop an analytical method for PAH metabolites in fish bile samples to enable us to understand PAHs metabolic pathways and their distribution in the marine ecosystem. For this study, ten PAH metabolites were investigated using GC/MS/MS A fast and efficient extraction procedure was developed in order to quantify the ten metabolites of naphthalene, fluorene, phenanthrene and pyrene in fish bile. Detection and identification of the analytes were achieved using gas chromatography coupled with tandem mass spectrometry. Deuterium-labeled hydroxy PAHs were used as an internal standard. The established method includes enzymatic hydrolysis, liquid-liquid extraction and derivatization. The recoveries were obtained from flatfish (79.8%-104.2%), marbled sole (85.7%-106.6%) and rockfish (88.3%-115.8%) in this developed condition.

TH138

Used of fish immune response in environmental risk assessment

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Growing awareness of the value of ecosystems is linked with the increasing demand by citizens and environmental organizations for cleaner rivers and lakes, groundwater and coastal beaches. This citizens demand is one of the main reasons why the Commission has made water protection one of its priorities. This explains the restructuring process of the Water Framework Directive (WFD 2000/60/EC) which concerns the general protection of aquatic ecology, specific protection of unique and valuable habitats, protection of drinking water resources, and protection of bathing water. Two elements are integrated in the ecological protection of surface waters: the "good ecological status" and the "good chemical status". Thus, the environmental objectives concern long-term sustainable water management based on a high level of protection of the aquatic environment and the introduction of the principle of preventing any further deterioration of the ecological and chemical status. In fact, the introduction of several environmental pollutants in freshwaters induces adverse effects in aquatic ecosystems by the disturbance of several physiological processes of aquatic organisms. This modification of the ecosystem could be avoided by increasing our knowledge on the chemical injuries induced on the main physiological functions, such as reproduction, maintenance, survival and behavior. In this way, we want to demonstrate the interest of fish immune response in environmental risk assessment using different river samplings.

TH139

Miniaturization of the salmonella/microsome assay in microsuspension

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The Salmonella/microsome assay is the most used mutagenicity test both for evaluation of chemicals and environmental samples. There are several versions of protocols available in the literature. Miniaturization of toxicological tests has been a tendency in compliance with the concept of the 3Rs (Replacement, Reduction and Refinement). MPF is a successful miniaturized version of the Salmonella/microsome assay, uses liquid medium and has a limited window of response. When quantification of the mutagenic response is important and strains with very different spontaneous reversion rates are used the assays has some limitations. Recently a protocol that uses 24 well agar microplates was developed and similar results were obtained when compared to the regular Ames test (plate incorporation version). The objective of this study was to miniaturize of the microsuspension Salmonella/microsome assay using agar microplates under the concept of the 3R and different strains with spontaneous revertants rates (low, TA1538, medium, TA98 and high, YG1041). Following the same procedure of the test Salmonella/microsome in microsuspension. Overnight cultures are concentrated 5-fold by centrifugation and resuspended in 0.015M sodium phosphate buffer. A volume of 50μL of cell suspension and 2μL of the sample are added in a tube and mix, then 13μL of this mixture are transferred for each tube test containing 12,5μL of 0.015M sodium phosphate buffer or S9 mix and are incubated at 37ºC for 90 min. To this new mixture, 500μL of molten agar are added and 200μL are poured into each well containing minimal agar. Colonies were counted after 66h incubation at 37ºC. Results were expressed as minimum effective concentration (MEC2), which is the concentration that provides the double of the spontaneous revertant rate. We are presenting data for TA1538 and TA98. Experiments with YG1041 are still being perfomed. Both protocols MPA and Microsuspension provided similar sensitivity for the strains analyzed so far. The MPA procedure is less laborious and uses less sample, materials and reagents. MPA procedure seems to be a promising tool specially to test environmental samples for mutagenic activity when quantity of sample is usually a limiting factor.

TH140

Chemical and effect screening of sediments in European estuaries: River basin specific pollution versus Europe-wide priorities

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In the frame of the Water Framework Directive (WFD) a good chemical status needs to be achieved in European rivers. The rivers are subjected to continuous discharge of various organic pollutants due to diffused and point sources (i.e. wastewater treatment effluents, agriculture and surface run-off). However, there is a general lack of knowledge regarding their occurrence and potential ecologic effects in different river basins. Disparate regulations of different countries and emission sources can also result in unpredictable scenarios in different European river basins. Therefore, a systematic monitoring of many emerging pollutants is not routinely applied and their environmental fate and ecotoxicological impact is still unknown. In order to assess the contamination patterns and the drivers of site- or estuary-specific effect responses, sediments samples of the estuaries of eight European river basins were collected. Sediments were selected due to their well-known role as a sink for hydrophobic persistent organic pollutants such as PAHs, PCBs or brominated flame retardants. However, in recent studies, it was suggested that also more polar compounds accumulate in sediments and significantly contribute to the general toxicity. The sediments were subjected to targeted chemical screening with high resolution LC- and GC-Orbitrap-MSMS of polar and non-polar organic compounds including for example classical POPs, pharmaceuticals, personal care products, surfactants, pesticides and biocides. Effect patterns were recorded using *Danio rerio* embryos applying a set of developmental endpoints together with specific biomarkers of toxicity including acetylcholine esterase activity, cyp1a and aromatase and behavioral assays such as locomotor response. Passive dosing was applied in order to simulate the exposure of fish embryos to the sediments. Preliminary chemical analysis of 15 pyrethroids and 19 PAHs show that there is a different distribution in different river basins. Also the bioassay results showed different toxicity patterns according to the sampling area. This approach will allow for multivariate analysis of contamination and effect patterns in order to group sampling sites and river basins and to identify the drivers of site- or estuary-specific response and thus supports the identification of River Basin Specific Pollutants.

TH141

Determination of Pesticide residues in sediment samples from Büyük Menderes River and Possible Risks for agriculture

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Pesticides are widely used because of the intensive agriculture. Runoff from areas treated with pesticides can pollute water. Pesticide residues in surface water can harm plants, animals and also ground water. Great Meandros River is very important for irrigation of surrounding fields in valley and also water system is used to deliver of river water to far away for ensuring irrigation in many crops. Meandros river was diveded in to 8 part in the same distance and 8 sediment samples were collected along the river. The samples were analysed by GC/MS-MS and LC/MS-MS. The most prevalent pesticides were detected 4,4 DDE, Fluvalinate, Formathion, Fenvalerate, Carbofuran and Pyrimethanil. A long term contamination of this pesticides could be expected by using them as irrigation water to the food web.

TH142

Effect-directed analysis (EDA) of Danube River water samples receiving untreated municipal wastewater from Novi Sad, Serbia

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The presence of endocrine disrupting chemicals (EDCs) in municipal wastewater has raised great concern regarding environmental health of aquatic ecosystem. Biologically active substances may cause harmful effects to human health and environment due to their potential to disrupt the endocrine system. The city of Novi Sad is one of the hot spots of pollution in the Danube River emitting untreated municipal wastewater into the river. The objective of this study is to identify EDCs and compounds causing oxidative stress responses in Danube river water influenced by Novi Sad wastewater through effect-directed analysis (EDA). Water samples from Danube River were extracted on-site using large volume solid phase extraction (LVSPE) into three different fractions i.e., neutral, acidic & basic with chromabond HRX sorbent (hydrophobic polystyrene-divinylbenzene copolymer). These extracts were pre-screened on GeneBLAzer bioassays (mammalian cancer cell lines) for agonistic and antagonistic hormonal activity for three nuclear hormone receptors (estrogen, androgen, progesterone) and one for adaptive stress response i.e., oxidative stress. Preliminary results showed highest estrogenic, antiandrogenic and oxidative stress potencies in neutral extract, which was selected for effect-directed analysis of chemicals causing these effects. Reversed phase-high performance liquid chromatography (RP-HPLC) fractionation was carried out with gradient elution by using C-18 silica based column (Macherey-nagel Nucleodur Gravity) with mobile phase methanol:water (50:50) with 0.1% formic acid. Two minute fractions (from 4-64 minutes) were collected (total 30 fractions) and reduced to dryness by an approach combining evaporation with solid phase extraction (SPE) on HRX. Biological analysis of these 30 fractions on GeneBLAzer identified several active fractions which are addressed with targeted and/or non-targeted analysis using LC-MS/MS.

TH143

Identification and Ecological risk assessment for genotoxic compounds in the Danube River

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One of the major challenges that environmental toxicologists and risk assessors are facing is the identification of exact toxicants in the complex exposure scenarios that are typical for many environments which we wish to protect. For that purpose, a linkage of effect data and hazardous compounds is required. In this study, bioassays, software simulation and chemical analysis were integrated to evaluate the potential ecological risk in the Danube River. The results indicated that samples from all three hotspots that were confirmed by fish embryo test (FET) induced significant genotoxic effects. According to the simulation of QSAR Toolbox and ChemProp, pesticides and PAHs were predicted to be the most genotoxic chemicals. Similarly, chemical analysis demonstrated that pesticide, especially 2,4-dinitrophenol, is the main genotoxicant in the Danube River. Ecological risk assessment by risk-quotient (RQ) method illustrated that 2,4-dinitrophenol caused medium level risk in the Danube River.

TH144

A snow/firn two-century record of polycyclic aromatic hydrocarbons (PAHs) and polychlorobiphenyls (PCBs) at GV7 (Antarctica)

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A two-century record of fourteen polycyclic aromatic hydrocarbons (PAHs) and seven polychlorobiphenyls\n(PCBs) was obtained from two snow/firn cores gathered at GV7 (Antarctica) in order to assess the contribution\nof human activities to global environmental pollution. The total concentrations of ΣPAH14 and ΣPCB7\nwere 1.2 ng/l and 0.09 ng/l in the deepest firn sample analyzed, and 3.4 ng/l and 0.22 ng/l in surface snow samples\ndeposited over the last fewyears, respectively. Our data highlighted the presence of PAH maxima in the concentration\ndepth profile, which correlated with non-sea salt sulphatemaxima associated with the major known\nvolcanic eruptions in the period 1800–1930. Between 1930 and 2002, PAHs showed an overall 50% increase, with a slope of about 0.013 ng l^{-1} $year^{-1}$. This\ncan be predominantly attributed to the emission of incomplete combustion processes of organic matter related\nto anthropogenic activities. PCBs show a much higher increase (+200%)with a slope of 0.0034 ng l^{-1} $year^{-1}$ in\na very limited period (1930–1980) which is almost totally due to the massive industrial production and use of\nPCBs, here named “industrial PCB excess”. The slight tendency of PCBs to a constant level from 1980 to 2012\nmight be attributed to the reduction in the industrial production of PCBs and the restricted use only in totally\nenclosed systems which started in many countries in the late 1970s.

TH145

Prioritisation of Pharmaceuticals in the Natural Environment in Kazakhstan

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Pharmaceuticals are readily available in Kazakhstan with most of them being freely available for purchase over the counter. Wastewater treatment systems in Kazakhstan are also old and employ old technologies so the treatment may not be as effective as in western countries. Consequently, emissions of pharmaceuticals to the natural environment in Kazakhstan are expected to be high and impacts could be greater than elsewhere in the World. However, unlike contaminants such as metals, oil and rocket fuels, which have been heavily studied in Kazakhstan, there are no data on the concentrations and impacts of these substances in rivers and streams in the country. In this poster, we present a desk-based study to prioritise active pharmaceutical ingredients of most concern in surface waters in Kazakhstan. Initially data were collected on the number of products and active ingredients for different therapeutic classes in use in Kazakhstan and on the typical doses and treatment durations. These data were then used alongside simple exposure modelling approaches to estimate concentrations of active ingredients (>500 active pharmaceutical ingredients from around 1800 products) in surface waters in Kazakhstan. For each of the active ingredients, the potential hazard to aquatic organisms was predicted based on human pharmacology data and also using quantitative structure-activity relationships. Risk quotients were then calculated for each pharmaceutical based on the exposure and hazard predictions and the substances ranked in order of risk quotient. In the future, experimental studies are planned in Astana, the capital of Kazakhstan, to characterise the concentrations of the top priority compounds in surface waters and to monitor whether impacts of these could be occurring in reality.

TH146

An integrated approach to assessing exposure and ecotoxicological impacts in the State of Qatar

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Exposure science and ecological risk assessment continues to develop and evolve in the State of Qatar. Efforts have been taken to utilize appropriate tools and methodologies developed in North America, Europe as well as other relevant hot and arid countries. In many cases, however, the extreme environmental conditions (e.g., high water temperature and salinity) and differing ecosystem structures (e.g., warm water species such as corals) require adaptation of the typical approaches and tools. At EMRQ we are developing an integrated approach to assess discharges, fate, exposure and ecotoxicological impacts in the marine environment. Our initial focus has been on the Liquefied Natural Gas (LNG) industry which is important to Qatar and other global regions; and increasingly so for North America and Europe. We have worked closely with our industry partners to robustly quantify discharges and model transport/fate using a spatially and temporally resolved hydrodynamic model combined with laboratory-based data. We also incorporate measured field data for evaluation of these tools and to assess ecological impacts. In the laboratory we have developed ecotoxicology protocols for reference species applicable to Qatari waters as well as dose-response relationships across various trophic levels and key contaminants. Current efforts include the development of an EcoRisk modeling framework which ties together these multiple components and environmental stressors into a tool that can be used by industry and government to assess ecological risk related to current discharge and exposure scenarios and also for future predictive and planning purposes.

TH147

Incorporating diffuse emissions in a load-impact based prioritization approach for metals

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In an effort to enhance public access to information, to examine progress in reducing emissions and, possibly, to help setting priorities for reducing emissions that may cause risk to man and environment, national and regional Pollutant Release and Transfer Registers (PRTRs) have been established. Currently, a series of metals are included in the reporting requirements that in first instance focus on point source and fugitive emissions at industrial sites. More recently the OECD debated about extending the PRTR schemes to include product and non-point source releases in order to make the pollutant registers possibly more relevant for risk prioritization and regional risk assessment. Indeed, PRTR registers are increasingly used for risk priority setting schemes under chemical management programs to ensure a more risk based rather than a hazard based prioritization. Currently, a main issue is that substances registers only include emissions (loads) making comparisons for prioritization less relevant if no correction is applied for impact (toxicity) potential. This poster will provide the outcome of a large exercise conducted by the metals sector (including Cd, Co, Cu, Mo, Ni, Pb, Sb, V and Zn) in the EU.. The metal loads at EU scale of non-point diffuse sources from products and other sources were assessed using up to date information and methodologies. Load-impact ratios were calculated as the ratio between the loads (t/y) and the metal specific chronic environmental toxicity reference values (e.g. HC_5). The relevancy of these load-impact results for risk-based prioritization approach and will be further discussed.

TH148

Degradation of Acetamiprid by esterase from aqueous solution
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Esterases are a group of enzymes which are the most important in the metabolism of xenobiotics including many pesticides. They protect the target site (acetylcholin esterase) by catalyzing the hydrolysis of pesticides.Acetamiprid is an systemic insecticide that is used to control many pests (e.g. Hemiptera, especially aphids) by soil and foliar application on a wide range of crops. The residue of acetamiprid is a big issue in many crops and countries. The aim of this study was to remove Acetamiprid in aqueous solution by addition of esterase enzyme. The process of acetamiprid removal from aqueous solution using esterase enzyme has been analyzed. Experiments were carried out at different time, different dose and pH to determine the optimum removing conditions. Analyses of acetamiprid residues conducted by LC/MS/MS. The performance of acetamiprid removal was found to be highly dependents on these conditions. Maximum removal was observed by esterase when compared by lipase and cellulase. Moreover our results will create opportunities for engineering degradative enzymes but more concentrations and mixture pesticides should be tested for further studies.

Identifying and regulating PBT and vPvB chemicals: Requirements,challenges and policy implications (P)

TH149
Bioaccumulation and PBT/vPvB assessment of organic substances: the inherent and unavoidable flaws in the current REACH approach
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Measurement of persistence, bioaccumulation and toxicity (PBT) properties is essential for identification of substances of very high concern. The assessment of the B criterion has challenges since it is currently based on relative rather than absolute metrics. This review focusses on the inherent flaws in some of the regulatory metrics for B, which are rooted in the low relevance of laboratory studies to environmental fate. Such a criticism is much less applicable to the measures of persistence and toxicity. Laboratory studies of bioconcentration/magnification factor (BCF and BMF), investigate minimal aspects of real environmental behaviour i.e. only uptake and elimination in very simple studies. More fundamentally, it cannot be avoided that these metrics depend strongly on exposure concentrations (e.g. high exposure in the diet can overwhelm the ability of the organism to depurate and confound meaningful measurements). The expression of, for example, BCF as a ratio of concentrations without reference to exposure, generates an illusion of steady state and a fixed metric. However, BCF considered as a ratio of *rate constants* is more useful. Therefore, the current system is internally flawed because a supposedly *intrinsic* property actually depends on exposure concentration, both in the lab and the environment. In reality, it is necessary to have a proper understanding of exposure route when considering uptake in the food chain. Yet the regulation is expressed without reference to exposure. Various authors have suggested metrics that are more robust measures of ‘B’ than BCF or BMF; only those which refer to rate are really useful. As far as possible, elimination rates should be based on environmentally-relevant exposures. What is the consequence of the flawed understanding of bioaccumulation metrics? Paradoxically it leads to the inconsistency that exposure has to be discussed when determining the hazardous properties! A properly-functioning regulation is needed because the regulatory consequences are serious: rigorous control of exposure and possible withdrawal from the market. The anomalies are particularly severe for ‘vB’. Some ways forward are suggested, based on meaningful measurements of elimination rates under relevant conditions; this would put B on a more solid basis, more consistent with P and T which have meaningful absolute units.

TH151
Monitoring, but for what? - Designing contaminant monitoring on a National scale.
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Whereas PBT and vPvB focused contaminant monitoring may serve to collect important field data for regulation of chemicals, the purpose of environmental monitoring is commonly also to inform about the state of the environment. The environmental monitoring run by the Norwegian Environment Agency are currently trying to do both, but are we doing it right? Traditionally, National contaminant monitoring programmes in Norway were designed to assess time trends in order to detect alarming levels and changes in levels of contaminants. In recent years, increased effort has been put in addressing how contaminants biomagnify in both aquatic and terrestrial Norwegian food webs. Such measurements potentially inform regulators on real world occurrence and fate of chemicals, and have shown to be a useful correction to lab-based measurements. Although valuable data already have been generated with this approach through Norwegian monitoring programmes, food webs fit for purpose has proven to be far from off-the-shelf. Whereas the model for fresh water biomagnification appears to work well, tools for marine and terrestrial food chains are more challenging. Moreover, other requirements for contaminant monitoring, such as implementing mixture toxicity risk assessment, evaluation of biological effect, and an ever-changing list of emerging contaminants for monitoring, leaves design of National monitoring a perpetual task. In the end, the question for regulators is therefore to what extent National contaminant monitoring should be focusing on emerging PBT and vPvB chemicals, compared to assessing the state of the environment.

TH152
Chemical policies, regulations and conventions in protection of European waters - Overlaps and gaps
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Several policy instruments (Regulations, Directives and Conventions), which regulate emissions of chemicals and use of chemicals in preparations and articles for reducing risks to the environment and human health by chemical pollution, have been reviewed with the aim to investigate how benefits can be gained by increased coordination, harmonisation and cooperation: EU Regulations - REACH, Plant Protections Products Regulation, Biocidal Products Regulation; EU Directives - Groundwater Directive, Marine Strategy Framework Directive, Drinking Water Directive, Urban Wastewater Treatment Directive, Sewage Sludge Directive, Industrial Emissions Directive, RoHS Directive, Toy Safety Directive; Multilateral environmental agreements - Stockholm Convention, CLRTAP, PRTR Theevaluation of potentials for synergies as well as risk for conflicts is carried out by a literature review, based mainly on information from the web sites of the issuers and from the legal documents and conventions, comparing how the following key aspects in WFD are treated in the other studied instruments: General characteristics: a) Scope: inland surface waters, transitional waters, coastal waters and groundwater; b) Reporting level: river basin; c) Substances Implementation: d) Characterisation of water bodies; e) Identification of pressures and impacts (emissions from point sources, emissions from diffusiive sources, establish inventories of emissions, discharges and losses); f) Monitoring programmes (concentrations); g) Status assessment of water bodies (Ecological status, Chemical status, Environmental Quality Standards; h) Programme of mesures; i) Reporting (river basin management plans) Early results indicate the potential for increased cooperation and coordination between e.g. WFD and REACH, for the safe management of chemicals, such as better sharing of information between the different instruments. Further analysis will address, e.g., procedures for including new substances, efficiency in implementing restrictions or bans, sharing of compiled information on occurrence, effects, properties and abatement options, specific sectors of society where chemical legislation needs strengthening. Future instruments development will be discussed to protect European waters against chemicals of emerging concerns and mixture effects– an innovative legislative framework also embracing novel risk assessment frameworks such as chemical footprints and planetary boundaries for chemicals.

TH153
OECD Test No. 309 (Aerobic Mineralisation in Surface Water) not applicable for a persistence assessment of strong adsorbing plant protection products with low water solubility
J. Hassink, B. Jene, BASF SE / Environmental Fate; D. Ebert, BASF SE
The OECD 309 study (Aerobic Mineralisation in Surface Water) is a new data requirement under EU regulation 1107/2009 for plant protection products, where compounds are incubated in an isolated natural water in glass vessels for 60 days with no contact to sediment. All experiences gained so far confirm that hydrolytically stable compounds will not degrade in this study because microbiological activity under thus artificial conditions is not sufficient to mediate significant degradation. The majority of the substances are required to be hydrolytically stable to be used by the farmer in spray application. In contrast to the OECD 309 study, the water/sediment study (OECD 308) is designed to provide at least a minimum of microbial diversity as existent in natural water bodies. It represents the most relevant worst-case exposure scenario of plant protection products, i.e. the exposure via spray drift, run-off or drainage of a small freshwater body with sediment at the edge of an agricultural field. The OECD 309 study might provide additional information for the risk assessment of a water soluble compound in coastal or oceanic water compartments but it is not appropriate to determine P-criteria for strong adsorbing substances with low water solubility by this study type, which is agreed by SANCO and ECHA guidance. The whole system DT_{50} of the OECD 308 is the only reliably estimated degradation half-life in a water containing system and therefore more suitable. Furthermore, photolytical degradation of a compound should be considered when relevant. As an example for low soluble and strong adsorbing substance, experimental data for the herbicide pendimethalin are outlined and discussed. Pendimethalin is hydrolytically stable and under the artificial conditions of the OECD 309 study (no light, no sediment) would exceed the P-trigger of 40 days for freshwater. However, in addition to the fast degradation in water by photolysis (DT_{50} 3.5 d), pendimethalin partitions within a few hours from the water into the sediment where it is rapidly degraded. Therefore, sediment is the only relevant compartment for pendimethalin in water/sediment bodies to be considered for the P assessment. The total system DT_{50} from water-sediment studies of 19.7 days is far below the P-criterion of 120 d for sediment. Aquatic mesocosm studies with

pristine sediment confirm the rapid partition of pendimethalin into sediment and its fast degradation.

Standards - an essential link between environmental science and regulation (P)

TH154

The Validation of Analytical Methods used in Ecotoxicological Studies

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The validation of analytical methods used in support of ecotoxicological studies has become a vitally important aspect of the registration process for plant protection products. The validation and reporting of analytical methods used to determine pesticide concentrations in ecotoxicological studies conducted for registration purposes are currently regulated by the SANCO/3029/99 rev 4. guidelines. These guidelines have been applicable under Dir 91/414/EC since revision 4 of SANCO/3029/99 was issued in July 2000. However, because directives such as this are generally not legally binding, in the past the guidelines were often either only partially followed or simply overlooked. When Regulation (EC) No. 1107/2009 came into force in 2011, adherence to the SANCO/3029/99 rev 4. guidelines became obligatory. This has resulted in serious implications for the registration process because ecotoxicological studies that had previously been accepted may well now be rejected on the basis of inadequate analytical methodology or incomplete analytical data. This is evidenced by the recent rejection of a number of residue studies by the authorities on the grounds of inadequate analytical methodology and/or data. It is therefore likely that ecotoxicological studies that fail to meet the analytical criteria required by SANCO/3029/99 rev 4. may not be accepted anymore. It is the purpose of this poster to give an overview of current requirements for analytical methods used in support of ecotoxicological studies. We also provide an example of a checklist that can be used to evaluate analytical methods according to SANCO/3029/99 rev 4. Simple tools such as this can be used to elucidate the potentially complex and time-consuming process of assessing the validity of analytical methods used in ecotoxicological studies.

TH155

Normalizing Data to the Control Creates Large Errors

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A technique common in the analysis of some ecotoxicity studies is to normalize data in treatment groups to the control. In the case of quantal data (e.g., mortality, emergence, hatching), the purpose is to adjust mortality data for background incidence and a method for doing this dates from 1925 called Abbott's formula. It has been known since at least 1985 that Abbott's formula can give biased results. Better ways to take background mortality into account have been known for several decades and software is widely available to carry out these alternative methods. This same idea has also been used for continuous responses, where the object is to estimate the concentration at which a specified percent effect relative to the control mean response occurs. The primary purpose in normalizing a continuous response to the control is to modify the data so that a probit analysis (which assumes quantal data) software package can be used. Good models are well known that do not require such data manipulation. It will be proved not only is the analysis of normalized data theoretically unsound and unnecessary, but can produce estimates as much as 6X too large or too small in several types of guideline studies while other readily available approaches provide accurate estimates. Theoretical problems with normalized data are first made clear. Second, data from fish early life stage, non-target plant, and fish sexual development studies are analyzed using probit models fit to normalized data and more appropriate models to indicate the difference in both ECx point and interval estimates. Third, Monte Carlo simulation studies based on experimental designs, variability and dose-response shapes typical of these studies are used to develop distributions for ECx estimates from these models. In the simulation studies, the true ECx is known, so these distributions can reveal the quality of estimates to be expected from the different modeling approaches. Both quantal and continuous data are considered Readily available software allows the use of statistically valid regression models to analyze quantal and continuous data arising from ecotoxicity studies. Analysis of normalized responses is unreliable, with both under- and over-estimation of ECx values by large factors occuring with little way to determine in a particular study that whether such mis-estimation has occurred.

TH156

COMPARISON OF NOEC VALUES TO EC10/EC20 VALUES, INCLUDING CONFIDENCE INTERVALS, IN AQUATIC AND TERRESTRIAL ECOTOXICOLOGICAL RISK ASSESSMENT

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Ecotoxicological studies performed for the authorization of plant protection products (PPP) usually result in the reporting of endpoint values in terms of effect concentration (EC) affecting a percentage x of test organisms or where a x percentage of an effect is observed (EC_x). The new Regulation (EC) No. 1107/2009 for the authorization of PPPs and the related data requirements provide that ecotoxicological endpoint data from chronic or long-term studies submitted by the Applicant are reported as EC_{10} or EC_{20} values together with the NOEC. NOEC values have been criticized since their values strongly depends on the experimental study design, whereas EC_x values take into account the whole concentration-response curve and are therefore considered more appropriate. The aim of the project is to investigate the comparability of the EC_x approach to the current NOEC approach on a larger data sets in view of the new Regulation requirements. Ecotoxicological data gathered from 70 active substances' approval dossiers were collected and stored into a MS Access database. All the extracted ecotoxicological data were analyzed in order to derive NOEC and calculate EC_{10}, EC_{20}, EC_{50} with confidence intervals, using statistical models from the exponential and Hill families for continuous data, and logistic, log-logistic and complementary log-log models for quantal data. The optimal model was selected based on likelihood ratio tests and the Akaike Information Criterion. EC_x/NOEC ratio distributions were calculated considering the whole set of data and model outputs; data were grouped in different categories to remark any differences in the EC_x/NOEC ratio distributions.

TH157

Robust data analysis to derive 17β-estradiol equivalent concentrations in in vitro bioassays

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Several different reporter gene assays that assess the activition of the estrogen receptor are currently being developed as ISO standards. Data analysis of these bioassay results is not a trivial issue and requires consideration to allow for comparable results between different assays or for the same assay but between laboratories.Typically the assays generate sigmoidal dose-response curves when a reference compound (or positive control, PC) or an environmental sample is applied in increasing concentration. Subsequently, dose-response curves of the reference and sample are used to derive equivalent concentrations for the sample. However, curves generated for the samples are often not parallel to those of the reference, or do not achieve minima or maxima values that are similar to the reference. Non-parallel dose-response curves may occur when the compounds in the sample are different from the standard, when antagonists are present in the sample, or when sample matrix interferes with induction in the assay. Maximum induction values may not be reached because of low activity of the sample, sample solubility problems or cytotoxicity. Low activity may be expected, for example, for surface and/or drinking waters. In these cases the type of data analysis that is applied will influence the equivalent concentrations that are derived. This makes the comparison of studies conducted in different laboratories challenging. To circumvent these problems we suggest using the 10% effect level interpolation ("PC10 approach") for *in vitro* data analysis. First, all data are normalized, where 0% refers to the response in the solvent control and 100% is the maximum response fitted for the reference dose-response curve. Then, the 10% effect level of the sample is interpolated from the reference dose-response curve and the 17β-estradiol equivalent (EEQ) concentration of the sample is determined. The requirements to apply the PC10 approach are: an effect level in the sample that is larger than 10%, or the ability to extrapolate the modelled sample response above the 10% effect level a limit of quantification (LOQ) that is below the 10% effect level. To discuss these specific aspects, we applied various calculation methods and show graphical examples of environmental samples that were assessed for estrogenic activity using different bioassays. The "PC10 strategy" appears to provide a harmonized solution for analysing *in vitro* screening data and a standardised data reporting practice.

TH158

Standards for specific in vitro assays - requirements for their implementation in regulatory frameworks

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Specific in *vitro assays* allow the assessment of the toxicological mode of action of a compound or the assessment of the toxic potential of an environmental sample. In recent decades, *in vitro* assays in ecotoxicology made a lot of progress because of their advantages such as rapidness, reproducibility, cost-effectiveness and their capability for high-troughput and automation. Although they are more and more accepted as alternative methods for animal testing up to now they are not widely implemented in regulatory frameworks. Still unresolved questions such as suitable mathematical methods for the derivation of effect-equivalence concentrations might hamper their broader use in regulatory frameworks for the aquatic environment. Effect-equivalence concentrations are used to express e.g. dioxin-like activity or estrogenic activity of a given sample as a concentration of a reference compound inducing the same effect-level in the assay. To compare different mathematical approaches for the calculation of effect-equivalent concentrations a set of standard conmpounds with known estrogenic activity were tested in several defined dilutions with the yeast estrogen screen (YES) – a bioassay that is currently being developed as an ISO standard. The selected compounds show specific concentration-response-relationships with e.g. different slopes. The assessment of the various methods for the derivation of equivalence concentrations is based on the assumption that e.g. a 1 to 2 dilution of a given sample should result in a bisected euqivalent concentration after testing with the YES. In case of the non-linear interpolation, a common approach for the calculation of equivalence concentrations, the calculated equivalence concentration depended on the maximal iso-NP-concentration tested in the YES. If a maximal concentration of 500 µl/l iso-NP is tested the calculated EEQ is about 60 ng/l. If the same solution is diluted 1 to 2 and tested again, the resulting EEQ is about 50 ng/l – the apparent EEQ of the undiluted original sample would then be 100 ng/l. The derived EEQ increases with dilution of the iso-NP. The same data will be assessed by several approaches under discussion for the derivation of the EEQ aiming to contribute to the development of a guideline for the derivation of effect-equivalence-concentrations.

TH159

Derivation of Environmental Quality Standards for biota using reliable bioaccumulation data

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The Water Framework Directive (2000/60/EC), or WFD, sets the strategy against the chemical pollution of surface water bodies. The evaluation of the chemical status allows parallel with the evaluation of the ecological status, to determine the overall quality of a water body. The environmental quality standards (EQS) are tools used to assess the chemical status of water bodies. Daughter directive (2013/39/EU) considers that EQS must be established in the matrix of the aquatic environment (water, sediment or biota) where a substance is likely to be found and where its concentration is most likely to be measurable. Some substances pose a significant risk through indirect toxicity (i.e. secondary poisoning resulting from food-chain transfer) and their analysis is more feasible in other environmental matrices, such as biota, then a biota standard may be required alongside, or instead of, the water column EQS. This work aims to derive quality standards applicable in biota equivalent to EQS for four dangerous and priority classified substances by the WFD (tributyltin, diethylhexylphthalate, short chains chloralkanes (C10-13) and pentachlorobenzene). Quality standards were derived using bioaccumulation factors. All retrieved literature was evaluated with respect to its relevance and reliability according to the TGD-EQS. Composition and completeness of the marine and freshwater food-chains are evaluated and only reliable and relevant data are considered valid for use in setting a quality standard. According to the matrix of the EQS, the principle of this deriving approach is based on: a conversion of the EQS from water to biota using bioaccumulation factors for each trophic level, or a conversion between different trophic levels (biota) directly by using the biomagnification factor if the substance is biomagnified, or by performing an intermediate conversion into the water if the substance is not biomagnified. Based on this principle, a general methodology for biota standards derivation is proposed. It seeks to determine, using a decision diagram, the best approach to convert a biota standard taking into account the availability of reliable bioaccumulation data. This methodology for deriving biota standards need to be validated for other substances in the context of monitoring of aquatic environments.

TH160

In flow cytometry, does results harmonization requires the same equipment? Case of cell viability

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Flow cytometry allow analysis of cellular parameters or functions in order to obtain statistical dimension both populational and individual. University of Reims Champagne Ardennes and INERIS, have set up a mobile environmental flow cytometry core facility: MOBICYTE. This core is dedicated to applications in the field of ecotoxicology, but also ecology functional and integrative ecophysiology. Among all our aim, we work on better understandings of technical confusing factors. More and more manufacturer proposed flow cytometry device particularly this 5 last years. All allow to analyze cell parameters but with different technologies. A lot of team works on same biomarkers, but some variation on protocol and method to analyze data are done. Based on this observation, we would like to harmonize the analyzed process from sample preparation to analyses raw data. First step was based on a consortium of six partners, all equipped with different manufacture flow cytometer, to assess the variability attributable to the equipment. In this study, a human monocyte cell line, THP-1, was chosen in order to have a homogeneous cell population to limit the variability of the measured responses. THP1 are exposed to set of increasing concentration of toxic agent, cadmium and cell mortality are revealed using a fixable fluorescent probe. Once labelled and fixed, cells pre-exposed to cadmium or not, are aliquoted and shipped to consortium for, analysis. Each laboratory analyzed sample set and determine both EC50 of cadmium and an unknown concentration. Raw data have been send to MOBICYTE for global analysis. For each set of sample analyzed on each cytometer, two data analysis was performed. The results obtained with the global analysis reveal a greater variability due to human component. Two immune functions are under study with same design: phagocytosis activity and oxidative burst.

TH161

Assessing the influence of part per billion variation of natural organic carbon levels on cationic polymer acute toxicity to D. magna

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Cationic polymers (CPs) are chemicals used widely for a variety of industrial and commercial purposes, for example, as floccing agents in water clarification. CPs generally have a low environmental concern due to the presence of organic carbon in surface water that greatly reduces their bioavailability, even at low mg/L levels. Although dissolved organic carbon can be as high as 50 mg/L in European surface waters, regulatory aquatic testing guidelines (OECD and EPA) limit total organic carbon (TOC) in clean dilution water to < 2 mg/L to determine the baseline toxicity of chemical substances. However the amount, source and quality of the organic carbon in dilution water can vary considerably between testing laboratories. To better understand the influence of minute TOC differences as a potential sources of variation for clean water studies, we explored the impact of 0.25, 0.50 and 1 mg/L additions of TOC (as humic acid) in artificial water (Elendt M4 medium) on CP toxicity, all of which are considered by definition to be clean dilution water. We chose *Daphnia magna* to assess the acute toxicity of CPs with varying molecular weights and cationic charge density. Our results indicate that part per billion levels of TOC produced strong CP toxicity mitigation. These results suggest that background TOC in dilution water is a potential source of variation and should be considered when interpreting the results of aquatic toxicity studies. Therefore, we recommend that aquatic tests used to establish the baseline toxicity of cationic polymers should be standardized with added TOC in the dilution water at a minimally measurable amount (1 mg/L) to quench low level variability of background TOC and represent an environmentally realistic worst case exposure.

TH162

Development of standardised methods in environmental toxicity assessment of nanomaterials

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In order to allow a reproducibly assessment of potential effects of pollutants a standardisation of ecotoxicity tests is necessary. While a range of standardised tests on environmental impacts of pollutants is available, their implementation in nano-toxicity testing is difficult and many of those methods are unsuitable for assessment of engineered nanomaterials (ENM). Small changes in environmental parameters or testing procedures can change the physico-chemical characteristics of the tested ENM and thereby influence the study results. In order establish guidelines and standard procedures for ecotoxicological assessment of ENMs, the NanoReg project aims to test, optimise and validate a set of suitable test methods for different types of ENMs. Preparation and characterisation procedures, a prerequisite for ENM toxicity evaluation, were established and validated for different ENMs in laboratories across Europe. Further, standard tests for toxicological assessments using the freshwater aquatic species *Pseudokirchneriella subcapitata* and *Daphnia magna* were evaluated and adapted to suit needs for ENM testing. A wide range of ENMs from various material classes are tested for their environmental stability and their potential ecotoxicological effects using the adapted ENM-standardised methods. The tested materials include different carbon based ENMs (native and modified single- and multi-walled carbon nanotubes, graphene and graphene oxide); a range of modified and unmodified titanium dioxide (TiO_2) based ENM, as well as metal ENMs. This large scale testing with suitable standardised methods will provide valuable ecotoxicological data and further show if a read across or grouping approach of similar ENMs is possible. Similarities between ENMs such as size, material and surface for potential read across or grouping approaches in aquatic toxicity tests can be identified.

TH163

Spill risks from Hazardous and Noxious Substances (HNS) - Is standard toxicity data enough?

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Over the last decades shipping of chemicals at sea have increased significantly leading to the need to be prepared to respond to incidents involving hazardous and noxious substances (HNS). Typically during chemical spills the impact upon marine organisms is estimated using spill model predictions of time-weighted average (TWA) or peak concentration compared to a predicted no effect concentration derived for constant exposure toxicity studies conducted in the laboratory. This study evaluates the toxicity of two chemicals, aniline and benzalkonium chloride, comparing standard and brief exposures. Salinity and temperature were also taken into consideration to understand the behavior of these chemicals under potentially different seasonal and regional conditions. Toxicity was evaluated for the copepod *Tisbe battagliai*, the red algae *Ceramium tenuicorne* and the brown algae *Fucus vesiculosus*. Benzalkonium chloride and aniline showed EC_{50s} in a similar concentration range (1-2 mg l^{-1}) based on standard exposures. When brief exposures were considered, contrasting effects for the two test chemicals were shown on all the species tested. Exposure to benzalkonium chloride resulted in significant effects after 1 hour exposure to a concentration of 5 mg l^{-1} but for aniline recovery of Tisbe (the most sensitive species to this chemical) occurred in exposures of a few hours at concentrations up to 300 mg l^{-1}. For variations in salinity and temperature toxicity decreased for some chemicals with the increase in salinity while increased temperature generally increased toxicity. Thus, a spill of a chemical with a specific mode of toxicity results in greater effects following relatively brief exposures, and exposed organisms show little or no recovery. In such cases impacts might be expected to be more extensive and persistent. In conclusion, for chemicals with specific modes of toxic action brief exposures, and possibly limited spill profile studies may be useful to support chemical spill risk assessment. Parallel studies on factors such as temperature and salinity are also valuable to help predict the likely scale of impact from chemical spills in different locations i.e. in the mouth of estuaries or further offshore or during different times of year or in different parts of the world.

TH164

RHIZOtest - the first standardized laboratory bioassay designed to assess the environmental bioavailability of trace elements to plants in soils

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A lot of laboratory bioassays were developed to assess the toxicity of trace elements on plants and among which some are widely used and even standardized at an international level. Surprisingly, there was no laboratory bioassay developed to assess the environmental bioavailability of trace elements to plants, that is to say the uptake of trace elements in plants. Considering the ecotoxicological relevance of the environmental bioavailability endpoint, we developed and tested a dedicated laboratory bioassay, the RHIZOtest, to allow its recognition and standardization it at an international level. The RHIZOtest is a plant-based test initially developed in the 1990s and used as a research tool to investigate the role of root-induced chemical processes as a driver of trace element dynamic in the rhizosphere and bioavailability to plants. The RHIZOtest is notably characterized the small size of the system and by a physical separation between plant roots and soil that enables to collect easily and quickly both compartments separately. These characteristics led to evaluate its performance as a risk assessment tool. We first assessed the robustness, the repeatability and the reproductibility of the RHIZOtest via an international ring-test. The RHIZOtest was hence validated for the measurement of the environmental bioavailability of arsenic, cadmium, cobalt, chromium, copper, lead, nickel, and zinc to three target plant species (tomato, cabbage, and fescue). We secondly assessed the ability of the RHIZOtest to distinguish the environmental bioavailability of trace elements for different plant species. Using a scoring approach, we were able to classify 10 plant species as a function of the bioavailability endpoint. Finally, we assessed the ability of the RHIZOtest to distinguish the environmental bioavailability of trace elements for plants grown in different soils. We thus exposed tomato, cabbage, and fescue to 55 soil samples exhibiting very constrasted physical-chemical properties. Armed with this knowledge, a draft describing the RHIZOtest tool and methodology was submitted to the international organization for standardization (ISO) and was validated as new standard, the NF EN ISO 16198, in February 2015. The development of the RHIZOtest is going on with its application to other contaminants such as nanoparticles and trace organic contaminants.

TH165

The leaf fatty acid composition of plants: new biomarker as a standard for assessing soil quality.

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The leaf fatty acid composition is proving to be a powerful biomarker to diagnose soil pollution. This biomarker is based on the fact that when plants are exposed to pollutants on soils, the reactive oxygen species (ROS) can increased dramatically and lead to cellular damages, including lipid peroxidation. The main target of lipid peroxidation is lipids containing polyunsaturated fatty acids, and more particularly linolenic acid (C18:3) mainly associated with lipid plastid membranes. We previously carried out numerous studies on the leaf fatty acid composition of lettuce grown under control conditions (*ex situ* approach) on contaminated and control soils and we showed that the %C18:3/(%C18:0 + %C18:1 + %C18:2) fatty acid ratio, called "Omega-3 index", decreased in leaves under stress. From these *ex situ* studies, it appeared that the Omega-3 index of lettuce leaves is an earlier bio-indicator than the standard tests existing in plant, such as emergence or growth inhibition. Moreover, we demonstrated that the Omega-3 index is a rapid, sensitive (metal and organic contaminants), robust, reproducible and repeatable biomarker of soil pollution which is then able to generate reliable data. These *ex situ* development of the Omega-3 Index led in 2012 to the publication by the French Agency for Standardization (AFNOR) of a soil quality standard (XP 31-233). We further studied this biomarker with plants grown on real sites during the French ADEME program "Bioindicators of Soil Quality". The results acquired during this program (among others) highlighted the efficiency of the leaf fatty acid composition of various plant species harvested on several areas of contaminated sites to provide an excellent biomarker of organic and metal contamination of soils. From these *in situ* studies, it was also developed and published a "mathematical" method with which it is possible in complex situations to analyze results provided by the use of the Omega-3 Index *in situ* and to rank different areas of the same site according to their phytotoxicity. Following these results, a standardization of the Omega-3 Index as a tool to assess soil quality through the International Organization for Standardization (ISO) goes underway with a desired applicability both ex and *in situ*.

TH166

Use of Standardised Methods and Risk-Based Approach in the Oil Industry

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A testing programme was implemented to investigate the management of produced waters using a risk-based approach. In employing this approach using standarised protocols, Whole Effluent Assessment (WEA) would potentially constitute an appropriate means of detection of unknown substances and has the advantage that each unique discharge is tested, rather than relying on predictive field toxicity values. Whole Effluent Assessment (WEA) was carried out on 15 platforms in the UK Continental Shelf as part of an assessment of the applicability of WEA as part of a risk based approach for the monitoring of produced water in the offshore industry. Installations were chosen to give a broad coverage of location, type of production (oil, gas), stage of production (early, late) and characteristic of produced water (oil content, water cut, use of chemicals and complexity of produced water) based on previous biannual data submitted. The UK study was carried out in parallel with other studies in Norway, The Netherlands and Denmark. WEA testing was carried out using bioassays from three trophic levels; bacteria (MARA and LumiMARA), algae (*Skeletonema costatum*) and crustacean (*Acartia tonsa*). WEA testing was carried out on produced water samples collected offshore at the same sampling point/time as samples were collected for biannual chemical sampling. Toxicity of the whole effluent during the RBA trial was found to be more representative of the risk compared with that of the individual substances. Thus was more indicative of the actual risk posed. Also the major contributing substances to the overall risk could be identified and the contribution of the natural components was determined to be significant. The study provided a comprehensive evaluation of the hazard and exposure assessment for characterisation of risk using bioassays and analytical techniques including modelling to formulate the UK RBA implementation programme. **RBA, WEA, Standardised, Risk**

A focus on research and education tools in environmental toxicology and chemistry (P)

TH167

PLATINAAE, an analytical platform for your research in environmental chemistry

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Ecotoxicological studies need high quality measurements of chemical contamination within all the compartments (physicochemical and biological) of the environment. In consequence, analytical laboratories must produce traceable analytical data, with high level of confidence, in numerous complex matrices. PLATINAAE (PLATeforme d'INgéniérie Analytique pour l'Agriculture et l'Environnement) is the central platform for analytical chemistry of INRA (French National Institute for Agricultural Research) mainly dedicated to plant and soil analysis. This platform is supported by two laboratories located at Arras (LAS) and Bordeaux (USRAVE). Both are equipped with high-performance instrumentation and are accredited according to the standard ISO 17025. In USRAVE, PLATINAAE works mainly on plants. It is capable of preparing and analyzing all parts of a plant (fruits, stems, roots, leaves, needles, wood...), and all types of plants (trees, cereals, grasses, fruits and vegetables...). The analyses concern the major elements essential to plant growth (nitrogen, phosphorus, potassium...), and trace elements, especially metallic trace elements with direct effects on the environment or health (arsenic, cadmium, lead, mercury...). In the LAS, PLATINAAE performs measurements on soils. The analysis done in this unit concern pedo-agronomic analysis (pH, particle size distribution, P, K, Ca, Mg, organic matter…), environmental analysis for metals (Cd, Pb, Hg, As…) and also for organic polluants (pesticides, herbicides, dioxins, PCB, PAH…). The determinations may be done as total content or as bioavailable content.

PLATINAAE works essentially as a partner for research programs. The main themes investigated are environmental contamination, biogeochemical cycle of elements in various ecosystems (crops, lands, forests...), soil quality, food chain or plant physiology. PLATINAAE performs also analyses on living organisms (invertebrates…) and wastes (sludge, compost…). In these scopes, the platform participates to numerous programs for environmental observation and experimentation, such as the ICOS Ecosystem program, the European program Biosoil, the RMQS network, RENECOFOR (monitoring of French forests)… PLATINAEE develops also innovative methods in analytical chemistry according to the needs of its partners (samples of very small sizes, trace and ultra-trace elements such as lanthanides or PGEs, mapping of plant organs…).

TH168

Biochem-Env, a platform of environmental biochemistry for research in ecology and ecotoxicology

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The consortium AnaEE-France (http://www.anaee-s.fr) aims at understanding and predicting the biodiversity and ecosystems dynamics in a context of global change. It will allow improving the understanding of biotic processes/environment interactions, mobilizing experimental and modelling platforms devoted to the biology of continental ecosystems, both terrestrial and aquatic. In this context, the objectives of the platform Biochem-Env (http://www.biochemenv.fr) are to provide skills and innovative tools for biochemical characterizations of soils, sediments, and micro-macro-organisms living in terrestrial and aquatic ecosystems. These approaches aim to assess ecosystems functioning. The scope of the platform Biochem-Env includes: -the technology intelligence and the development of tools used for the biochemical characterization of solid environmental matrices and macrofauna, -the development of an Environmental Information System (referential for functional biodiversity) for managing the traceability of samples and data, and improve our ability to characterize the biological status of environmental matrices. Available as a scientific partner in the frame of collaborative research projects, the platform's abilities ranges from advice providing (sampling, protocols and data analysis), to technical training, including analysis and experimentation in regional, national and international research programs. The tools of the platform have been deployed in programs targeted at assessing the sensitivity and restrictions of biological indicators of soil quality, subjected to various constraints (waste landfilling, farming practices, contaminated sites and soils…). For example, long-term experimental sites provide opportunities to assess the effects of pressures and threats on soils and their functioning. In a context of soil contamination, we can conclude using global soil enzymatic activities that soil functioning is enhanced in biological farming comparatively to conventional practices.

TH169

MOBICYTE: mobile cytometry in environmental risk assessment

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Commonly used in biomedical science, flow cytometry is more and more implemented in environmental sciences allowing studies on numerous cell types such as bacteria, yeasts, phyto / zooplankton and invertebrates and vertebrates cells. Environmental cytometry requires adaptations of techniques in cellular biology. Developments of field methodologies are required in order to study cell organizations and functions closed to their interactions with environmental variables and environmental stress in wild contexts and with taking into account of samples quality preservation. In this context, the mobile environmental flow cytometry core facility named MOBICYTE is dedicated to applications in numerous scientific fields and particularly in environmental toxicology and ecotoxicology, but also in functional ecology and integrative ecophysiology. This core can be used by research teams as well as by public and private organizations working in environmental biomonitoring programs. Our mobile equipment is operable in the more isolated environmental field contexts (aquatic and terrestrian), considering cell models from various animal species (algae, mollusk, fish, wild mammals…). Some studies using MOBICYTE platform will be described in order to present new opportunities for biomonitoring the health status of wildlife organisms in health and environmental risk assessments contexts.

TH170

Analysis of 13-year soil monitoring data of Korean Soil Quality Monitoring Network

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The Korean soil quality monitoring network has been operated since 1987 and has yearly reported concentrations of soil trace elements for 1,521 observation points. This study analyzed 13 year soil monitoring results (1997-2009) of Korean soil quality monitoring network. The monitoring results were reported with concentrations and land uses for 9 trace element concentrations (Cd, Cu, As, Hg, Pb, Cr^{6+}, Zn, Ni, and F). This study analyzed tolerance limits of the trace element concentration data for each observation point. This study classified 278 points as "variation occurred", showing that the upper 95% tolerance limit of the variation occurred point data was greater than the Korean soil quality standard. This study also evaluated anthropogenic contamination of those points by deriving background concentrations from the total soil quality monitoring data. Analysis results showed that fluoride levels were frequently varied in Korean soils and industrially-used sites showed significant changes in trace element concentrations. However, this study found that high nickel concentrations of soil monitoring data were attributed to naturally high background concentrations. This study also suggested better soil quality monitoring network strategies for achieving sustainable soil environment. The most important strategy for producing reliable soil monitoring data is to sample soils at the consistent points every monitoring time. (This study was supported by the GAIA project (2014000560001) funded by Korea Environmental Industry & Technology Institute and Korean Ministry of Environment.)

TH171

"Métatox": a new one-year training program for engineering students on assessment and management of toxicological risks for both human and ecosystem health

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In recent years, the global contamination of our environment has generated a major challenge: understanding the long-term impacts of multi-exposures to a multitude of chemical substances at low doses. Current regulations evolve with the increase of concerns regarding the impact of anthropic activities on both human and ecosystem health. Today, these concerns are essential for citizens, politicians, managers and firms. As a consequence, all industries have now to integrate their impact on the environment and the human health, since the assessment of the innocuousness of a substance or product, towards both humans and ecosystems is now a part of the evaluation of manufacturing processes and development of new products. In this context, AgroParisTech, the leading French Graduate School in Agronomy, Environment, Life science and Food technology, has created a new one-year training program for engineering students that develop a holistic approach of toxicological risks for humans (environmental and food toxicology) and ecosystems (ecotoxicology) health. "Métatox" is one of the specialized programs offered to the students as a last year of their Master of Engineering; it is co-organized with Anses (French Agency for Food, Environmental and Occupational Health & Safety) and Ineris (French National competence centre for Industrial Safety and Environmental Protection). "Métatox" students get in-deep knowledge and skills on the impacts of contaminants on living organisms (including humans) and ecosystems, with an integrated, multidisciplinary approach. "Métatox" offers the necessary background in chemistry, biology, ecology, statistics and modelling to understand the fate of chemicals in the environment and the mechanisms of their toxic effects at different levels of biological organization. In addition to this fundamental knowledge, practical teachings initiate the students to the methods and tools covering the full range of risk analysis: risk assessment, management and communication, especially in the framework of the European regulations (i.e. REACh, EU Water Framework Directive, Biocides Directive). With this interdisciplinary course and systemic approach, the future engineers will both fully analyse a wide type of risks, and propose efficient actions and management strategies to protect human and environmental health.

TH172

Petnica Science Center: A place for engaging science education in Southeastern Europe

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Petnica Science Center (PSC) is an independent, non-profit organization for science education in Serbia. PSC has over 35 years of experience in organization of programs for gifted high school and university students, as well as educational and outreach programs for science teachers. Among the 17 programs at PSC available to students, over 40% are related to biology, chemistry and environmental sciences. Nearly 2500 alumni from over 450 academic institutions and companies, together with PSC`s team of trained professionals, form our network of associates, lecturers and peer educators, all of whom are involved in course design and who help put on our numerous activities and programs. Each year, 1500 students from Serbia and Southeastern Europe attend programs at PSC. Since a major reconstruction completed in 2012, PSC currently represents the most modern center for extracurricular science education focused on young people in Europe. PSC houses fully equipped state-of-the-art laboratory facilities for biology, chemistry, and physics. PSC also houses a scientific library with 40.000 books and journal acces. PSC also provides full board accommodation options for up to 170 participants at a time. A multidisciplinary approach is the hallmark of all PSC activities, which requires efficient integration of all our laboratories, instruments, students, and associates. Specific facilities include a laboratory for cell cultures and molecular biology, a center for confocal microscopy, HPLC-MS and GC-MS equipment, a laboratory for analyses of trace elements equipped with an AAS and XRF, plant growth chambers, as well as several 3D printers and a 3D scanner, all of which are situated in one central laboratory building. The characteristics of PSC as a unique venue led to major changes in the concept of the most recent Young Environmental Scientists (YES) Meeting, organized by the Students of SETAC. The 4th YES meeting was hosted by PSC in March 2015, and for the first time in a YES meeting we offered hands-on short courses for 130 meeting participants. All 8 courses took place in one day simultaneously for all students. PSC's laboratories equipped with cutting-edge instruments and our network of dedicated alumni and associates and partners make PSC one of the most convenient and engaging venues to host hands-on courses, summer schools, and scientific conferences related to current topics in environmental science.

How can we improve the link between academic research and policy-making in order to advance chemical risk assessment and management? (P)

TH173

Regular information of stakeholders and policymakers about a soil monitoring of persistent organic pollutants in Cuba

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Although Cuba has signed, ratified and put into force the Stockholm Convention on Persistent Organic Pollutants (POPs, 2008), there exists no systematic monitoring tool that would reflect and assess the environmental and human exposure for chemicals. Cuba has been active in the Strategic Approach to International Chemicals Management (SAICM). In a project from 2010 to 2011 funded by SAICM, the United Nations Environment Programme (UNEP) and the Global Environment Facility (GEF) POPs were monitored globally (including Cuba) and passive air samplers were installed to evaluate the exposure. With our project, Soil-Q (Establishing a soil monitoring network to assess the environmental exposure to polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in the province of Mayabeque, Cuba), the investigation on POPs in Cuba has been reactivated by monitoring the soil. The newly established soil monitoring network in Cuba currently consists of 39 sample locations in the province of Mayabeque, and additional 12 stations in the adjacent province of Havana. From these sites, representative soil samples were taken, using the rigorous protocol of the Swiss Soil Monitoring Network (NABO). Analytical methods for the quantification of PAHs and PCBs have been established in the laboratories of the Cuban partner. Results indicated low rural exposure with these pollutants (max. 110 μg/kg of the sum of the 16 PAHs regulated by the EPA, max. 5 μg/kg of the sum of the 7 non-dioxine like PCBs). In contrast, concentrations at some places in Havana are considerable and mandate remediation. Workshops were held (initial and to present the above results) to decision makers (representatives of the agricultural, energy, environmental, and chemical safety sector). Present were also members from the Swiss Embassy and the Swiss development and Cooperation in Cuba (COSUDE). The echo and interest in the audience was high because Soil-Q covers the urgent demand of Cuban policymakers and stakeholders to know the POP pollution in their soils. The Cuban TV was also used for the dissemination of first results and an explanatory website about Soil-Q and its long-term aim is planned at the Cuban partner institute of agriculture (CENSA). Soil-Q is as a promising start to advance in the chemical risk assessment and management and has, as stakeholders and decision makers were approached from the beginning, a high potential for future research and related dialogues.

TH174

An Analytical Framework to Evaluate and Ameliorate Science-based Policy-Making in relation to complex environmental concerns using the latent constructs, Legitimacy and Effectiveness

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The effectiveness and legitimacy of science based policy-making in Europe for the endocrine disruptor 17α, ethinyl-estradiol (EE2) is challenging to evaluate. Traditional policy performance measurement tools predominantly target regulatory compliance downstream of the policy development process. A dearth of tools to evaluate upstream policy processes such as scientific risk assessment and legislative proposal development hampers analysis of the legitimacy and effectiveness of policy-development processes. The use of science to guide policy decisions is hence rarely, systematically evaluated or monitored. Consequently, understanding of causes and potential solutions to effectiveness and legitimacy deficits is severely curtailed risking sub-optimal policy outcomes and loss of trust in both governing authorities and the scientific expertise upon which they rely. The present study characterizes specific challenges of environmental risk policy interventions with respect to EE2 thereby facilitating recognition of the following issues confronting policy-makers: i) scientific & economic uncertainty in relation to adverse effects, their consequences and potential mitigation and management options, ii) low-consensus deliberative processes typified by values and interests divergence, iii) high complexity derived from social pluralism, institutional diversity and inter-regulatory dependencies. An analytical framework is derived pursuant to an extensive literature review and operationalized via a qualitative study in order to facilitate a systematic evaluation of variables impacting effectiveness and legitimacy in the case of EE2 policy interventions. The legitimacy of deliberative processes, the implementation thereof and concomitant participant discourse is evaluated and the effectiveness of resulting policy outcomes discussed. More specifically, the discourse of participants to deliberative processes at key decision points in the policy development process is analysed. Variables influencing the development of policy-makers' perception of risk (constructivist risk), the role of science therein and predominant cognitive mechanisms employed to transpose scientific evidence into decision outcomes are thereby identified. Understanding of the challenges of applying & using science and its relative influence on outcomes of policy-making processes is thereby greatly enhanced.

TH175

Socio-Economic Impacts of Copper Compounds Classification in Industrial Sites

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Regulation 1907/2006/EC concerning the Registration, Evaluation, Authorization and Restriction of Chemicals (REACH), offers risk management measures to assure safe use of dangerous substances. Regulators use socio-economic and environmental considerations to assess impacts of applying those measures. However, it remains a challenge to properly assess the effects of risk management measures in several industrial sectors. Currently, the integration of the cost and benefits of applying harmonized classification on substances is not included in the decision-making process. The implementation of harmonized classification has direct and indirect consequences for industrial sectors and ecosystems such as the provisions to transport the substances. This research uses an integrated economic-environmental analyses to assess the trade-off of applying harmonize classification in industrial sectors. It includes provisions for organizational and investment cost and, as a novelty, incorporates the external environmental cost and benefits, based on ecosystem services quantifications. The integrated method is applied in several case studies based on recent regulatory decisions that impact the European Copper industry. The results show that the environmental benefits of applying the measure cannot overcome the side-effects and the costs for industrial sites in a twenty years period.

TH176

MACRO-DB: A Risk assessment tool for management of pesticide use in water protection areas in Sweden

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In Sweden, farmers are not allowed to use plant protection products on agricultural fields located in water protection areas for abstraction of drinking water, without permission from the municipality. The risk of a pesticide leaching to groundwater or surface water is dependent on several factors and their combined effects cannot be easily predicted. There are 290 municipalities in Sweden and the assessments are performed in many different ways sometimes resulting in a high degree of inconsistency in the outcome of the decisions. This causes differences in the level of protection of the drinking water in Sweden as well as unequal conditions for the farmers, which causes conflicts between farmers and the local authorities in many municipalities. The Centre for Chemical Pesticides (CKB) at the Swedish University of Agricultural Sciences has developed a risk assessment tool, MACRO-DB, to help the municipalities to assess the risk of pesticide contamination of the drinking water. MACRO-DB is based on the MACRO model which is a one-dimensional, process oriented, dual-permeability model for water flow and pesticide transport in agricultural soils. MACRO-DB is a simplified, user-friendly tool in two steps, mainly aimed for environmental inspectors, farmers and agricultural advisers. The first step is a very simple web application based on worst case scenarios. In the second step there are a limited number of parameters that the user needs to identify to run the model; site specific information on soil properties, crop, active ingredient, dose and time for application. Predefined sets of parameters for each regional climate, soil class, crop type and pesticide are provided in the tool, as well as pedotransfer functions for soil hydraulic properties. The results are given as a simulated concentration in the water, at 2 m soil depth, which will reach the groundwater and a simulated concentration in the water leaving the drainage system, which will reach the surface water. MACRO-DB was released in September 2013 and the tool is now used in many municipalities in Sweden. MACRO-DB makes it possible for the local authorities to base their decisions on best scientific knowledge. The results can also act as an independent, common foundation for discussions between the different stakeholders, which can help preventing unnecessary conflicts.

Advances in Social Life Cycle Assessment and Life

Cycle-based Sustainability Assessment (P)

TH177

Communicating results in a life cycle sustainability analysis

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We developed an approach to life cycle sustainability analysis (LCSA) where the research questions are case-specific and defined in a participatory process. The LCSA results are likely to be a mix of quantitative modelling output and qualitative discussions on widely divergent topics. Communicating such complex results is a challenge. We applied the LCSA approach to the case of a 50 km pipeline for transfer of residual heat from industries to a large district-heating system. The LCSA included 14 research questions on economic, environmental and social aspects. We communicated the results not only in a report, but also in a powerpoint presentation with one slide for the response to each question. The content of the slides ranged from bar charts to bullet points. To make the presentation more uniform, we used a happy, neutral or sad smiley on each slide. The conclusions were summarised in a table with the smiley and a very brief comment for each question. We found this presentation efficient for communicating our interpretation of the results. However, it did not allow for communicating the methodological choices, assumptions etc. that are necessary for the audience to assess our results and conclusions. Instead, we saw a tendency for the audience to accept the smileys without further reflection. In conclusion, smileys are highly efficient for communicating conclusions on widely divergent issues, but they do not create a good basis for discussion of the results and analyses. This means they might close the door for debate over the decision at hand. This is a problem since our participative LCSA approach is particularly relevant for cases that greatly concern several different stakeholders, and debates are important to resolve conflicts of interest. Hence, communicating not only the conclusions of an LCSA study but also the complexity of the study remains an important challenge.

TH178

Sectorial analysis of the current state of life cycle sustainability assessment in the Chemical Industry: insights from the MEASURE project

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The Horizon 2020 SPIRE Program has developed a clear vision for the future of the European process industry for a more sustainable, competitive and growing economy. Ambitious reduction targets have been formulated for energy and raw material intensity and for greenhouse gases emissions. Cross-sectorial collaboration along the value-chain is key to achieve these objectives. Harmonization of methods for life cycle sustainability assessment (LCSA) along the value-chain is necessary to allow a consistent quantification of the overall benefit of the alternative strategies developed within the SPIRE program. In this context, the European project MEASURE (Metrics for Sustainability Assessment in European Process Industries) was launched aiming to address critical issues and gaps in sustainability assessment. A sectorial analysis was conducted for several process industry sectors. In this poster, special attention is given to the sector Chemistry and Consumer Goods and challenges for the implementation of LCSA. Overall, a good degree of consistency is observed in this sector concerning life cycle indicators used. The WBCSD Guidance Document "Life Cycle Metrics for chemicals" (2014) is seen as a useful fundament. Nevertheless, some topics were identified as critical issues leading to remaining inconsistencies. Industries face many challenges for assessing (eco)toxicity due to unresolved methodology issues, low data availability, results' sensitivity and practical interpretation. Currently, toxicity issues assessed via LCA are rarely included in the decision-making process and play a minor role despite the recognized relevance of the indicator itself. The assessment of bio-based products remains also a critical issue: biogenic carbon is often inconsistently considered over the life cycle, the calculation of the emissions from Land Use Change are lacking harmonization, while other aspects such as the impact on biodiversity or land quality are poorly covered. Recommendations to tackle some of these issues will be presented to improve the implementation of LCSA. Finally, industries of this sector have gained experience in assessing sustainability along the innovation process thanks to R&D adapted tools, and a staged assessment process starting with the use of screening tools based on selected indicators and ending up with a full Life Cycle Analysis. Such approach will be presented and recommended to assess projects and their innovations in the SPIRE context.

TH179

Development of Life Cycle Social Impact Assessment Method Using Covariance Structure Analysis

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In recently years, Social Life Cycle Assessment has been paid attention. In 2013, UNEP/SETAC Life Cycle Initiative published a report about S-LCA, which describes a list of subcategories and candidates of indicator. However, there is very little information regarding cause–effect chain models indicating the relationship between inventory and impacts in society. The development of a method for S-LCA with clarification of cause-effect chain is critically important to improve the quality of assessment. ISO37120-Sustainable development of communities-was standardized in 2014. This provides a list of indicators for sustainable development in city service. There are a hundred of indicators with classified into seventeen themes. WCCD (World Council on City Data) published statistic data that meet with the indicators listed in ISO37120. Therefore, this study attempts to develop a framework of S-LCA method using statistic data which characterize social aspects of cities in the world. We use covariance structure analysis. This study draws Social Impact Pathway based on covariance structure analysis result.

TH180

Safe and Sustainable Chemicals, from question to method

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Developments such as depletion of resources and climate change urge a transition to a sustainable economy. But what is sustainability? A popular way to evaluate sustainability in terms of impacts on man and the environment is life cycle analysis (LCA). A drawback of LCA is the extensive amount of data needed and environmental, health and safety (EHS) aspects are generally not fully taken into account and are not compatible with substance regulations. Just as important is the necessity for defining the sustainability question to be answered. In many cases the choice for a method is based on habit or nice of expertise and not made explicitly. The aim of this study was to obtain insight in sustainability assessment methods currently used for or applicable to chemical products, biobased compounds, and production processes of substances, to structure these and organise it into a sustainability assessment identification key. We developed this identification key for manufacturers, policy makers and those who perform sustainability assessments. The applicability of this approach is shown in a case study. Four cases were chosen, two feedstocks and two products: organic waste, sugar beet, chemical building blocks and bioplastics. Stakeholders were selected, interviewed and brought together within workshops to discuss their views on sustainability, the use of LCA methods, and knowledge gaps. An overview of the stakeholders' questions regarding sustainability will be given and the application of the identification key is illustrated. In this way a transparent translation of theory and aims (definition of sustainability and promoting sustainable development) is put into practice, which is an often-missing link in current sustainability case studies in literature. *Acknowledgement* - This research is carried out in the framework of RIVM Strategic Programme (SPR), in which expertise and innovative projects prepare RIVM to respond to future issues in health and sustainability. We gratefully acknowledge the Athena Institute of the VU University Amsterdam for sharing their expertise and assisting in the stakeholder analysis and participation.

TH181

Screening of sustainability indicators for conventional renewable energy systems

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Sustainability indicators are increasingly important to decision- and policy-makers. Within the framework of the EERA Joint Programme on Economic, Environmental and Social Impacts of Energy Policies and Technologies ("EERA JP e3s", www.eera-set.eu), a specific sub-programme working on "A life-cycle approach for evaluating the sustainability performance of energy technologies" was launched in 2013. The goal of this sub-programme is to develop and harmonise indicators and methodologies used to evaluate environmental, social and economic impacts of energy systems. Within this context, a review of more than 100 articles and reports on the evaluation of energy systems was carried out. The strategy followed for the identification of appropriate sustainability indicators focuses on analyses at the sub-sector level. This work complements previous studies on bioenergy systems by screening sustainability indicators for conventional renewable energy systems: hydro, geothermal, solar and wind power systems. After removing recurrent indicators, 150 indicators reported in the literature were evaluated and screened according to the following criteria: life-cycle perspective, practicality (availability and reliability), and relevance (specificity to the sub-sector assessed). A reduced set of indicators was therefore defined, not only covering the three sustainability dimensions (environmental, economic, and social) but also including multi-dimensional indicators (e.g., techno-environmental and socio-economic indicators). The selection of environmental-related indicators was found to be significantly affected by the specific sub-sector under assessment, in contrast to social and economic indicators.

TH182
Identifying sustainability indicators through Open Space
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Sustainability is a broad concept with a large number of environmental, economic and social aspects. Any sustainability assessment can cover a selection of these aspects only. The choice of aspects and indicators is vital, since it can heavily affect the conclusions of the assessment. Different aspects will be crucial in different case studies. A case-specific approach that involves stakeholders in the selection of assessment indicators is likely to increase the perceived relevance of the results and the chances that the results of the assessment are accounted for in the decision process. The Open Space technology is well suited for discussing and deciding how to organise an activity where the structure is not known in advance. It has been used for, e.g., organising a conference, addressing a local societal challenge, and developing an action plan for a non-governmental organisation (NGO). The Open Space format for workshops is designed to create ample opportunities for meetings and discussions, where each individual participant has the freedom to join and leave discussions whenever he/she chooses. Individual deliberations and small-group discussions alternate with reporting and discussions in plenum in order to generate and preserve the energy in the meetings. Deciding on the topics and the schedule of the workshop is part of the workshop itself. We organised Open Space workshops at the initial stage of a life-cycle sustainability analysis (LCSA), inviting concerned companies, NGOs, policy makers, and researchers. We found the output from the workshop useful as basis for prioritising among the sustainability aspects and for formulating research questions for the LCSA, although this required an interpretation of the workshop results. We also found it necessary to add further research questions to make the LCSA sufficiently comprehensive and balanced. A key challenge in LCSAs with Open Space workshops is to obtain a sufficiently large and representative group of participants in the workshop. This is likely to be possible primarily in cases that greatly concern several different stakeholders, such as policy decisions or investments in infrastructure.

Challenges in data analysis, weighting, valuation and visualisation - How to enable decision makers to make trade-offs while being transparent for all stakeholders (P)

TH183
Exploring feasibility of monetarization and water footprint
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In a context where stakeholders ask for understandable results to support reliable decision making and communication, the subject of monetary valuation is prevailing. Moreover, companies began to notice increases in their exposure to risks (environmental and business risks) due to several reasons: access to resource, increase in price, supply disruptions, lower quality of resource, competition for resource allocation between stakeholders including local population, etc. Life Cycle Assessment and Footprint metrics do not appear to be very intuitive to decision makers used to monetary values. Monetarization is seen as a valuable way of expressing LCA and footprint results but still needs to be investigated further. This work, jointly carried out by Veolia R&I and CIRAIG, aims to investigate possible applications of monetarization to water footprinting. Work is based on the Water Impact Index methodology from Veolia (Bayart et al., 2014). First, a critical review of the literature on the issue of monetarization of potential impacts and risks associated with water has been conducted aiming to put into perspective the different approaches. The term impact includes in particular resource depletion and loss of ecosystem services, while the term risk here means business risk associated with environmental impacts. Second, a critical analysis of the limits and scientific relevance in a context of multi-criteria decision has been performed. Finally, preliminary recommendations on how monetarization can be applied to water footprint are suggested. Keywords: Water Footprint, Water Impact Index, Monetarization, Weighting. References: Bayart, Jean-Baptiste; Worbe, Sébastien; Grimaud, Julien; Aoustin ; Emmanuelle. 2014. The Water Impact Index: a simplified single-indicator approach for water footprinting. *The International Journal of LCA*. Vol 19; p. 1336:1344.

TH184
The Ecological Scarcity Method: Deriving Country-specific Environmental Targets for Definition of Critical Flows
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The Ecological Scarcity Method (ESM), also called Ecopoint Method, is based on a distance-to-target approach: it defines critical flows which denote a target level for selected physical flows to the environment, and compares these flows to the current respective flows, leading to so-called eco-factors. Critical flows and current flows are to be specified for defined system boundaries, usually the national level, which for the original ESM is Switzerland. The ESM has been applied in the framework of company environmental management of the Volkswagen AG (VW) since 2001. At VW's German sites the ESM has been adapted to German framework conditions in the course of a research project. As a next step the ESM shall be transferred to VW's international sites. However, this poses specific challenges for which a methodological approach has been worked out. The relevant eco-factors were first allocated to the related environmental problem and grouped according to the level in terms of global, regional or local impacts. For each of these groups, which were allocated based on the scientific basis of the concept of Planetary Boundaries, a preferred conceptual framework for identification of environmental targets has been proposed resulting in the Top-down-, Spatial- and the Bottom-up-approach. As a second step a comprehensive research of data bases, statistics and literature in order to assess data availability for critical as well as current flows for the proposed approaches modified the grouping from environmental problems to the level of impacts. Data related to global environmental problems belonging to the Top-down-approach were collected from international organisations. For the spatial- and bottom-up-approach data was drawn from international data bases or accessed from national organizations. From this research, a viable procedure for identification of environmental targets for each eco-factor was identified. For the issue of energy consumption procedures for down-scaling national targets from an international perspective have been worked out and compared to national policy goals. Results show the outcome of policy strategies on environmental targets and related critical flows.

TH185
Comparative life cycle assessment of alternative solutions for the management of medical equipment discarded: the case of a panoramic X-ray dental machine
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In 2012 in the European Union there were 11,000 hospitals and 68,000 medical institutions (including long-term care facilities, clinics, specialized clinics, laboratories, etc.), with about 3,000,000 beds for acute illnesses. The supply of healthcare services is highly dependent on the use of medical equipment, whose employ is continuously increasing. Moreover, the use phase of the life cycle of medical equipment is very short. In fact, the adoption of new standards, the need to improve safety and functionality of the equipment and also marketing purposes contribute to a continuous renew of the medical equipment: It is estimated that in European hospitals a medical equipment is averagely used only for 5 years. This brings to an increasing amount of medical equipment disposed. Once disposed, most of the medical equipment become a WEEE, i.e. a Waste of Electrical and Electronic Equipment, which represents both a serious risks for the environment, primarily due to their content of hazardous materials, and at the same time, if properly managed, a valuable resource. Considering healthcare institutions, scientific literature highlight that not enough attention is payed to medical WEEE, which are often neglected, stored in basements or in unused premises or donated to charity organization for their shipment in poor Countries without any warranty on their real destiny. Therefore, it is very important to promote a proper management of medical WEEE within healthcare institution. In this work, a comparative life cycle assessment is performed to assess the environmental implications of different realistic solutions for the end of life of a panoramic X-ray dental machine. This work is part of the Life-MED project (LIFE13 ENV/IT/000620), funded by the European Union through the Life financial instrument.

TH186
Public decision support & LCA: feedback from testing a simplified LCA tool for wastewater systems
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The environmental efficiency of a wastewater treatment plant (WWTP) is usually assessed through its effluents quality. But this quality is achieved at the expense of other impacts occuring during the construction, operation and dismantling of the whole wastewater system (WWS) including sewers and WWTP. Many studies performing LCA of WWTPs already exist, but there is a lack of simplified and operational tools usable by non-specialists of LCA to perform LCA of entire WWSs. The objective of this work was to develop a simplified software providing objective environmental indicators to be included in the decision making process along with other criteria when chosing among WWSs options. To reach this goal, specifications for a simplified software were defined and a first version was implemented (namely ACV4E software). The potential users are public

wastewater services and engineering consultancies. The LCA results should be presented to local authorities for decision making and in some cases to citizens to justify the choices made. ACV4E was applied to real cases, to test the software as well as the appropriation process and the effects on decision making. In addition a working group involving stakeholders was created. This group made a focus on the interpretation of LCA results, with the challenge of making them understandable and usable for non-specialists of LCA. The experiment suggests that the co-construction of the calculator with potential users is crucial for appropriation. It partially opens the LCA black-box and allows to better meet field needs. However, classical mid/endpoint bar charts proved to be inefficient for non-specialist interpretation and for communication to politicians and normalisation of the results almost always leads to misinterpretations. As a result of the working group process, these classical charts will be replaced by new types of representations. Local authorities that tested ACV4E were very interested in this tool even if the environmental criterion was often secondary compared to other criteria in a decision context (like compliance with legal standards and financial aspects). Finally, this experiment concludes that such a tool was more difficult to use directly by local authorities for which the need for LCA in decision making for WWSs choices is not frequent enough. Engineering consultancies could be the main users of the software because they have technical skills and will have more regular use of it.

TH187

Interpretation of LCA results for GRP pipe systems through normalization lenses

V. Vladimirov, HOBAS; M. Pazdro, REICHHOLD AS

HOBAS and REICHHOLD took a significant step in direct collaboration of both product R&D and life cycle assessment. The study evaluates the different choices of unsaturated polyester resins (UPR) for cc-GRP pipe systems, from a LCA perspective. To this purpose three types of resin have been used to manufacture HOBAS products and the environmental impacts were compared. The resins are: (a) Standard UPR, (b) UPR resin containing recycled PET material (denoted rPET-UPR) and (c) UPR resin containing bio-sourced material (denoted BIO-UPR). Based on EN 15804:2012 (Sustainability of construction works) the study compares fourteen impact indicators for pipe systems in the three scenarios. After the results were obtained and interpreted, normalization was applied in order to cross-check the first understanding of the indicators. CML data from April 2013 (Leiden University, CML-IA, Version 4.2, April 2013) was taken and the total emissions for Europe "EU25+3" (Wegener Sleeswijk et al., 2008) were used as the reference scenario. For example, for a product manufactured with BIO-UPR resin in the first step increased variation of "renewable energy", "water consumption", "stratospheric ozone depletion" and "photochemical ozone creation" were noticed from the calculation. The results of the second step, normalization, indicate that the impact category with by far the most relevance is the "depletion of abiotic resources". Therefore, the issues which could be considered for further analysis may concern the use of crude oil (i.e. the formulation of the BIO-UPR resin includes intermediates made from crude oil). Thus, choice of BIO-UPR resin is a rather complex process and can hardly be reduced to the simple question of, for instance, which resin generates more or less CO2-eq emissions for the product. The use of normalization is of great help in identifying possible next steps in product R&D. The BIO-UPR formulation used in this study is one possibility of many. Based on the LCA results, focus can be put on elements that matter most. For the polymer industry the development of alternative and renewable raw materials represents a key task. This study represents a step ahead on the path to sustainability!\n\n

TH188

Allocating an environmental load to wastewater sludge when sludge moves from "waste" to "product", a methodological challenge

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Wastewater sludge is currently considered as a waste but with new industrial practices and european regulation (End-of-Waste directive) considerate will be soon a valuable product. This paradigm shift must be followed by the consideration of environmental constraints to introduce this new product on market. Assessing the environmental performances of these future 'marketable sludges' can be processed with Life Cycle Assessment (LCA) but it first implies to take into consideration the whole life cycle of this new product from the raw material extraction up to the product end of life. How this can be reasonably done when waste LCA consider that the waste is free of any environmental load when entering the system, assumption often known as the "zero burden approach"? Giving a environmental load to "product sludge" consists in the resolution of several methodological questions which arise as the wastewater treatment plant will provide several coproducts : the clean water and the sludge, both having a merchandable value. This paradigm shift implies that the wastewater treatment plant has several function and multifunctionnality has to be resolved. Because sludge is a direct and indivisible production from water treatment, process subdivision cannot be achieved. In the same basis, system expansion can be barely used as there is no known alternative to the "wastewater treatment" if system is expanded to only keep the "sludge production" one. Finally, allocating an environmental burden to these two coproducts appears to be the best way to solve the induced multifunctionnality. As a consequence, allocation factors have to be developed to allocate an environmental burden both to the sludge produced and to the clean water released in the receiving media. This is quite challenging as it supposed (i) to model the sludge production both from primary treatement and biological treatment, (ii) to create new allocation factor based on these physical and biological processes as classical allocation factors cannot be used, (iii) to validate these allocation factors whatever the wastewater treatment plant configuration. This paper aims at presenting the allocation factors used to charge sludge with an environmental burden when sludge is from now considered as "product sludge".

TH189

Integrating LCA and risk assessment for decision support

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In the last decades, the use of classic decision making theory has gained a lot of attention as decision support tool. Indeed, when stakeholders and governments face the problem of decision making over public safety and health or sustainable development, their judgment has to be justified to the community by objective proofs. In this perspective, sustainability and safety are the major issues to be considered in decision making. Decision makers always face the dilemma that the most sustainable solution may not be the safest one. Therefore, it is a challenge to optimize the system, ensuring sustainability and at the same time minimize risks. The Global Decision Support Initiative (GDSI) is a cross-disciplinary center established at the Technical University of Denmark (DTU) aiming to develop a new framework model for decision support with associated tools to solve this problem. Firstly a Life Cycle Assessment (LCA) is conducted to screen the studied system and identify the hotspots where emissions are largest and hazards are more likely to occur. Decision analysis is then applied to optimize the alternative that may reduce the consequences caused by those hotspots. To start with, we first identify and characterize the potential hazards and then quantify their associated direct and indirect consequences for each hotspot. A finite number of available actions are chosen among the ones that can reduce the consequences. For each action we apply decision analysis to evaluate the effect of the actions in mitigating the consequences, expressed in failure cost and damages to human health. Meanwhile, we apply LCA to assess the environmental impacts changes caused by the action. A common metric is developed to allow comparing RA and LCA results on a common scale and eventually integrate them into one result. The result is then used to rank the alternatives, taking stakeholder preference into consideration, and the optimal solution is identified. Here the traditional LCA is applied in the framework of decision making together with RA. It does not only identify the problem, but also provides ranking of possible solutions, where consequences from both potential risks and environmental emissions are considered. This framework is flexible and can be broadly used to support decision at different scales, e.g. national, sector or product level.

TH190

A normalisation and weighting procedure (ESA) to assists the decision making process: The case study of salt mining in the chlor-alkali industry

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Life cycle assessment (LCA) is a powerful tool to assess the potential environmental impacts throughout a product´s life-cycle [1]. However, LCA results comprises several impact categories that makes difficult the process comparison [2]. To reduce the LCA complexity, this work proposes a normalisation and weighting procedure to carry out the environmental sustainability assessment (ESA) of the extraction and purification of salt in the chlor-alkali process. The ESA is based on two main variables: natural resources sustainability (NRS) and environmental burdens sustainability (EBS) [3]. Different variables play an important role in the quantitative estimation of the NRS, mainly the materials, water, and energy usage need to be considered. Three main EBS are involved depending on the natural environment: air, water, and soil and related to the emissions, effluents, and wastes leading to different environmental impacts. Nevertheless, these functions are rarely normalised thus remained as functions rather than variables. This methodology suggests the normalisation of EBS based on the threshold values proposed in the European Pollutant Release and Transfer Register E-PRTR, and a similar procedure based on the average consumption of natural resources of the process under study for the normalisation of NRS. Moreover, the normalised indicators can be subjected to a weighting procedure to obtain a global index that will help the decision making process. However, the use of an absolute index could potentially mask certain results. For this reason, the comparison of several processes by means of a global index can be used to obtain an overview of the environmental performance of the system and to optimise the process. Nonetheless, individual indicators should be also assessed to determine the main environmental problems and thus provide opportunities for improvement of the critical points of the process.

TH191
Representativeness of environmental impacts in relation to Life Cycle Inventories
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Life Cycle Assessment is based on Life Cycle Inventory (LCI) of systems that identifies all emissions to the environment and consumptions of resources (1,800 referenced environmental flows in the Ecoinvent database). LCIs show many sources of variability due to the diversity of systems, the model uncertainties, the variations of the real world... Following the construction of LCIs, emissions and consumptions are translated in terms of environmental impacts through a set of impact categories. Different Life Cycle Impact Assessment (LCIA) methods (ReCiPe, TRACI, CML, Eco-indicator...) propose different ways of characterizing categories and each of them can be used to determine the results on the set of impact categories modeled. Then, LCIA methods reduce the complexity of systems comparison from a 1800-dimension space (i.e. the number of substances emitted or taken from the environment) into a less than 20-dimensional problem (i.e. the impact categories) where each dimension has an environmental meaning. Each impact category represents, to a more or less precise extent, the original LCI. The aim of the present work is to determine how far impact categories are relevant. The method automatically organizes impact categories according to the information on LCIs that they gather. LCA results interpretation could be helped by this development by indicating the impact categories for which the LCI is the most correctly represented. The 11,332 LCIs of the Ecoinvent 3.1 database and a total of 692 impact categories were used in the present work. Both of them belong to a vector space of 1,769 dimensions where each dimension represents an environmental flow. After a data pre-processing (i.e., data cleaning and data normalization), cosine squared between directions of LCIs and impact categories were computed. The higher a cosine is, the more the impact category direction is simillar to the LCI direction, and the more correctly the impact category considered represents the contribution of each environmental flow. Cosine squared results for each impact category were compared by using their median value. Grouping categories by their environmental meaning shows tendencies from eutrophication potential to land occupation/transformation for their ability to accuratly represent LCIs from Ecoinvent.

TH192
Promoting Innovation & Technology in Europe: Sustainability Support and Information Network of Infrastructure
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In the roadmap to a resource efficient Europe, it is indicated that a sustainable and guaranteed supply of raw materials will be essential, together with an efficient transformation and use of the resources. The EIT (European Institute of Innovation and Technology) Raw Materials will contribute to this challenge, but to boost effective and efficient resource management in the EU, insight and support is required in sustainability of virgin resource extraction, recycling, resource conversion and environmentally, socially and economically sustainable technologies in general. The SSIC (Sustainability Support and Information Centre) and facilitates sustainable technological development and educational activities by making use of a state-of-the-art sustainability assessment toolbox. This Network of Infrastructure (NOI) project brings partners together with complementary expertise and therefore is the central contact point for industry and technology developers, from resource extraction to product design and recycling, when it comes to questions regarding input-output analysis, life cycle assessment, life cycle sustainability assessment, socio-economic assessment, sustainability of material flows and stocks, cost benefit analysis, recyclability benefit calculations, criticality analysis, supply disruption etc. It can be consulted in up-scaling projects, other NOIs, education, training etc. but will also be leveraged outside the EIT Raw Materials and will link up with existing initiatives at European level. Offering a fast and effective access to state-of-the-art datasets, methodologies and tools, the SSIC delivers a service package at the highest level for innovation growth in Europe. While looking for opportunities to strengthen the consortium by networking and linking to other initiatives, fostering collaborative projects with SSIC and external partners, the consortium will add through a multidisciplinary approach in making raw materials a major strength for Europe.

Poster Corner Abstracts

Contaminants of Emerging Concern in the Environment and their Management (PC)

MOPC01
Pesticide contributions of urban effluents and storm sewer on a French peri-urban river contamination
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Currently, pesticides are widely used by different users such as farmers, private consumers or public authorities. These compounds can be released into the environment and contaminate water resources, potentially impacting aquatic ecosystems. Several contamination routes are well identified: diffuse losses due to agriculture uses, or direct releases because of urban effluents. Storm sewers and wastewater treatment plants (WWTP) are known to be vectors of pesticides. Indeed, several studies have shown the presence of pesticides in wastewaters; moreover WWTPs are not able to remove all of them efficiently without adequate tertiary treatments. The purpose of this work was to study a continuum including a wastewater network, up to the collection natural aquatic ecosystem, a peri-urban river, receiving treated WWTP effluents and untreated inputs via storm sewers. This has allowed us to identify the main contributors of pesticides to the river contamination. It has been shown that the major source of pesticides used in agriculture is the upstream of the studied system, as expected, mainly due to metabolites of metolachlor (0.2 $g.d^{-1}$), one of the most used herbicide on the territory. On the contrary, WWTP effluent appeared to be the major vector for pesticides mainly used for urban purpose such as fipronil (0.5 $g.d^{-1}$) a veterinary used insecticide or diuron (2. $g.d^{-1}$), an herbicide added in paintings for building protection against mosses and algae. Domestic wastewaters are suspected to bring more pesticides than industrial ones because some pesticides are classified as biocides and are mostly used by private consumers such as fipronil. Storm sewers have been shown to be also a source of contamination, but at a lower level than WWTP effluents. Such integrated studies by documenting the presence, sources and fate of pesticides all along a continuum could give paths for reflection and *in fine* actions on sources, to reduce pesticide contamination of natural surface waters. *Acknowledgement* This study benefited from funds in relation with RESEAU and "Plan Micropolluants" projects (associating as partners: Bordeaux Métropole, SUEZ, IRSTEA, Water Agency, CNRS, University of Bordeaux, CHU and Aquitaine Regional Council). This work was funded by the Aquitaine Regional Council and SUEZ. This study has been carried out with financial support from the French National Research Agency (ANR) in the frame on the Investments for the future programme, within the Cluster of Excellence COTE (ANR-10-LABX-45)

MOPC02
Pollutant fluxes in small catchments: Impact of urbanization, flow dynamics, and pollutant degradation
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Urban areas are responsible for the release of numerous emerging contaminants into the environment. Wastewater-related compounds are constantly introduced into aquatic systems by wastewater treatment plants (WWTPs). Hydrophobic chemicals such as PAHs are mainly released during flood events and the related combined sewer overflows. Within catchments, rivers act as transport pathways. While some pollutants undergo attenuation, others are very persistent. In this study emerging and particle related pollutant fluxes are assessed for the Steinlach and the Ammer rivers, two tributaries of the River Neckar in SW-Germany. For dissolved pollutants (e.g. pharmaceuticals and personal care products as carbamazepine, diclophenac or triclosane), fluxes have been estimated based on various sampling campaigns including monthly samplings and high-resolution sampling during base flow and flood conditions. By use of Lagrangian type sampling at consecutive control sections, also the persistence of these pollutants could be assessed. In water samples collected during event samplings, a correlation of total concentrations of hydrophobic pollutants (PAHs) with turbidity and/or total suspended solids was found, which allows for the calculation of pollutant concentrations on suspended solids. For both catchments, such correlation coupled with continuous monitoring of discharge and turbidity enabled the calculation of particle-bound pollutant fluxes. The results showed that fluxes of wastewater-related pollutants are preferably influenced by consumption patterns and WWTP efficiency. During flood events, these compounds tend to get diluted, revealing a source limitation of their input into the environment. Among these compounds diclofenac shows the highest fluxes in the monitored rivers. However, it must be reminded that dissolved compounds can undergo natural attenuation processes. In that regard, their fluxes decrease with distance from the source. Carbamazepine – as a counterexample – also shows high fluxes but behaves conservatively and may spread along farther distances. Results of on-line turbidity monitoring showed that high turbidity/discharge events account for the majority of particle-related pollutant loads. In contrast to soluble compounds, the mass fluxes are positively correlated with discharge, yet practically cease during base flow conditions. On the long run, however, both types of pollutant fluxes may be relevant and should be monitored to a sufficient level.

MOPC03
Modified-zeolites as potential sorbents for removal of per- and poly-fluoroalkyl substances from aqueous environments
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Perfluoroalkyl substances (PFASs) are a class of stable chemicals used in fire-fighting foams, industrial surfactants, insecticides and surface treatments for paper and textiles. Perfluorooctane sulfonate (PFOS), its salts and perfluorooctane sulfonyl fluoride (PFOSF) were added to Annex B of the Stockholm convention on persistent organic pollutants (POPs) in May 2009. A number of sites, such as landfills, agricultural sites and sites treated with biosolids (sewage sludge) have been contaminated by PFASs. PFASs have also been found in groundwater. Limited work has been done on PFAS removal from aqueous environments. Sorbents (e.g., activated carbon, zeolite, peat etc.) for conventional organic pollutants might not be effective for remediation of PFAS. Knowledge of sorption processes and appropriate material types (i.e., sorbents) for PFASs is limited. Also most recent studies have only focussed on PFOS and PFOSF. In our study, we modified a natural zeolite (clinoptilolite) to provide four sorbent materials and investigated their sorption potential for nine PFASs. The results based on the 24-h batch adsorption test were promising. CPC-zeolite and DDDMA-zeolite are the most effective among the four modified-zeolites in removing PFASs from aqueous environment. The sorption ranks were PFOS > PFNA > PFHxS > PFOA > PFHpA > PFBS > PFHXa > PFPA > PFBA. The modified sorbents had higher concentration removal for long-chain than for short-chain PFASs. For the same number PFAS carbon atoms, sorbents had higher sorption of PFSAs than PFCAs. This research is on-going. We continue to investigate sorption kinetics, temperature effects, maximum sorption capacity, sorbent dosage effects, particle-size effect, PFASs competition effect, as well as the stability of these sorbents and the leachability of sorbed PFASs. Keywords: Perfluoroalkyl, sorption, modified-zeolite, aqueous. *Acknowledgement* – Thanks to the Natural Sciences and Engineering Research Council of Canada Grant # EGP477520-14.

MOPC04
Human exposure to plasticizer chemicals - correlation between indirect and biomonitoring exposure estimates in a Norwegian human cohort.
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Plasticizers used as additives in numerous consumer products have a continuous release into the environment (pseudo-persistent), leading to consecutive human exposure which might cause adverse health effects (e.g. endocrine disruption). Indoor air quality and house dust may have significant impact on human health because people spend most of their time indoors, where the concentrations of plasticizers are comparatively high. Inhalation and dermal exposure through air, transdermal exposure through dust adhered to the skin, oral exposure through dust ingestion (hand to mouth contact) and diet have been considered as major exposure pathways. On the other hand, daily intake of plasticizers can also be determined through analysis of urinary metabolites. In the present study, indoor stationary air, personal ambient air and house dust (floor and elevated surfaces) samples were collected, extracted using solid phase extraction (SPE) and/or microwave assisted extraction and analyzed by GC-MS/MS. Based on the resulting concentrations of the diesters in these external media, we calculated the daily human intake rates for the Norwegian cohort, and compare them with reference tolerable daily intakes (TDI) for each analyte. Alternatively, the human biomonitoring approach allowed us to assess to which extent plasticizers entered the bodies of 61 adults living and working in the Oslo area (Norway). Finger nails and three intraday urine spots were collected from each participant in order to

monitor their internal concentration. The analysis of nine phthalate metabolites, together with two DINCH metabolites in urine and nails was performed by LC-MS/MS and used to back-calculate the initial total exposure to the parent compounds. Among phase I metabolites, high levels of the monoesters of DEP and DnBP were observed in nails (median concentrations of MEP 31.2 ng/g and MnBP 21.4 ng/g), while in urine the median concentrations of the more specific biomarkers of secondary metabolic oxidation for DEHP were OH-MEHP 4.5 μg/L and 5-oxo-MEHP 4.7 μg/L, and for DINCH were cis-OH-MINCH 0.5 μg/L and cis-cx-MINCH 0.5 μg/L. The correlation of indirect exposure estimates and human biomonitoring exposure estimations is under investigation.

MOPC05

Method development for non-target screening of sewage sludge using comprehensive two-dimensional gas chromatography coupled to high-resolution mass spectrometry (GCxGC-HRMS)

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More than 100,000 chemicals are present in the technosphere and 30,000 of these are considered to be so-called "every-day chemicals". Sewage treatment plants (STPs) are used to remove nutrients, but also some metals and organic chemicals, from urban waters and create a less contaminated effluent. Consequently, STPs form a link between the technosphere and the environment. A by-product of the sewage treatment process is sewage sludge – a solid product that contains nutrients as well as pollutants. These nutrients make the sewage sludge attractive for applications as fertilizer on agricultural fields, provided that the contaminant levels are not too high. In order to be able to investigate pollutants occurring in sewage sludge an analytical method for comprehensive non-target screening is needed. Up till now there is no method existing that allows a full screening of sewage sludge for a wide variety of compounds with different properties, which is the ultimate goal of this project. The current work has aimed at developing a validated non-discriminating sample preparation method for gas chromatography - mass spectrometry (GC-MS) analysis. Pressurized liquid extraction (PLE) was used for extraction, with in-line and/or off-line clean-up. In the in-line approach silica gel was added to the PLE cell for simultaneous extraction and clean-up, whilst the off-line approach involved clean-up by gel permeation chromatography. Analysis was performed by comprehensive two-dimensional GC (GCxGC) coupled to high-resolution MS in both cases. Good recoveries were obtained for all tested compounds with either one or both of the proposed sample preparation methods. Therefore the combination of both methods allows a comprehensive screening of sewage sludge contaminants by GC-MS.

MOPC06

Illicit drugs as new class of emerging contaminants in aquatic systems: high specific level of contamination in French West Indies compared to global monitoring results

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Since 2013, authors analysed each year illicit drugs (19 molecules and metabolites related to cocaine, cannabis, amphetamines, opiates and substitute consumption) in urban wastewater inlets of treatment plants (WWTPs) of Fort-de-France, the main city of Martinique island of French West Indies. Caribbean are the hub of cocaine trafficking and a hotspot of cannabis production. This area is also a hotspot for biodiversity, including several patrimonial species and ecosystems, on which the effect of emerging pollutant residues is unknown. Wastewater were daily sampled during a normal week, i.e. without festive event or other confusing factor, following a protocol framed by the European Union's programme SCORE, gathering now worldwide similar initiatives. Samples were analysed (solid-phase extraction on Oasis HLB cartridges then LC/MS-MS quantification) after SCORE inter-calibration agreement. Results show very high concentrations of cocaine and metabolites in comparison to published results,, exceeding 3 μg/L for benzoylecgonine (BZE) and 1.5μg/L for 11-nor-9-carboxy-delta-9-tetrahydrocannabinol (THC-COOH): this levels are ten-fold higher than those published for other cities. Opiates and their substitutes and amphetamine-like compounds were never detected. This data confirm a Caribbean-specific profile of drug consumption, partly explained by the place of this archipelago in the international market of drugs. Considering to temperate environment, some results begin to demonstrate ecotoxicological effects for European or North-American species at environmental concentrations, i.e. tenfold less than results from Martinique. Moreover, severe leakage of sewer and non-collective sanitation threaten surface water. Combining the small watercourse of the river and the local high population density, coastal and marine ecosystems could be rightly be considered as exposed to such molecules, in combination with pharmaceutical residues. Authors will discuss the potential input of illicit drugs on the aquatic environment in Martinique Island, the predicted values regarding to actual data on ecotoxicological effect of the studied molecules, and the mean to ward off the urgent need for determining the stake of such emerging micropollutants on the accelerating biodiversity loss observed in patrimonial ecosystems.

MOPC07

Toxicity of the biocide and plastic additive Triclosan to marine organisms: environmental risk assessment

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The 5-cloro-2- (dichlorophenoxy) phenol, or Triclosan, is a biocide of general spectrum widely used in multiple pharmaceutical and personal care products (including tooth paste) and also as an additive to different kinds of plastics. We have tested the toxicity of this substance by means of three standard marine bioassays representing sensitive and phylogenetically distant biological models, including the microalgae *Isochrysis galbana* , nauplii larvae of the copepod crustacean Acartia and embryos of the echinoderm *Paracentrotus lividus*. In increasing order of toxicity, the EC_{50} (μg/L) for sea-urchin embryos was 149 (95% CI: 140 to 158) the LOEC 120 μg/L and the NOEC 100 μg/L. For *Acartia* nauplii the EC_{50} was 94,3 (95% CI: 88,73 to 99,79) and the EC_{10} was 64,8 (95% CI: 53,69 to 75,81). For the microalga the toxicity was even higher, with a EC_{50} of 34 (95% CI: 28 to 40), a LOEC 20 μg/L, and a NOEC of 10 μg/L. These results allowed us to quantify the risk posed by Triclosan on coastal ecosystems. The results also suggest that Triclosan concentrations should be surveyed in coastal waters, and water quality standards implemented for this substance.

Microplastics in the environment: Sources, Fate and Effects (PC)

MOPC08

Occurrence, composition and adverse effects of microplastics in native mussels collected along coastal and marine areas of the northern Adriatic sea

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In the recent years, the occurrence of microplastic particles in the aquatic environment has gathered a rising scientific interest. Several studies proved that the ingestion of microplastics may negatively influence the physiology of marine organisms of different feeding strategies, particularly in those species which can not discriminate the food source. Recent studies have posed the attention to the accumulation of these particles in the food web and the direct consequences to the human heath as even larger amounts of seafood are consumed nowadays. On the other hand, evidences also suggest the role of microplastics as vectors of chemical pollutants either used as additives during the polymer synthesis, or adsorbed directly from seawater i.e., PAHs, PCB, surfactants etc,. However, models simulating physiological conditions in the gut suggest that both adsorbed pollutants and chemical additives of plastics might be released to organisms. Despite the importance of microplastics in adsorption and transport of hydrophobic pollutants, little is known about the distribution, accumulation in the marine food webs as well as their direct and indirect harmful effects. On this context, the Adriatic Sea represents a semi-enclosed basin characterized by a low water recirculation rate and an elevate anthropic pressure associated with unsustainable fishing and high inputs of environmental contaminants. Thus the primary objective of this study was to investigate the body burden accumulation, polymers characterization and eco toxicological effects in native blue mussel (*M. galloprovincialis*) collected in various marine areas along the northern Adriatic sea. Results were compared to data collected from native populations of coastal areas. The early outcomes both show a significant site-, time- and oceanographic conditions-related distribution and support the needing of a seafood safety monitoring program to better understand the actual human health related risk.

MOPC09

Microplastic particles analysis by Raman micro-spectroscopy and chemometrics: morphological and chemical characterization

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World production of plastics has been steadily increasing since 1950, reaching 311 million tons in 2014. This results in an accumulation of plastics in the marine environments. The global spread of microplastics requires the establishment of standard analytical methods to distinctly identify them and estimate their abundance in all environmental matrices (water, sediment and biota). Most studies have used visual sorting to rank a particle as a microplastic but this method presents a high probability of a misidentification, especially with decreasing particle size. To date only a few studies on the detection and chemical composition analysis of microplastic particles have been published. Several methods have been developed to identify plastic polymers such as vibrational spectroscopy like micro-Fourier-transform infrared (FT-IR) spectroscopy and

Raman spectroscopy, which are non-destructive methods, and sequential pyrolysis-gas chromatography coupled to mass spectrometry (destructive method). The main disadvantage of these methods was the analysis of only selected subsamples and not the whole sample. In the present study, an alternative method was proposed to rapidly analyze all particles in a sample with Raman micro-spectroscopy. Three laser wavelengths (633, 691 and 785 nm) and three substrates (glass fiber filter, nitrocellulose membrane filter and gold coated microscope slide) were tested to increase the ratio between Raman intensity and fluorescence background. The analysis of particles was carried out using a combination of static image analysis of particles and automated Raman micro-spectroscopy allowing the counting, size, shape and chemical composition of a large number of particles. The optimal combination was the 785 nm-laser with the gold coated microscope slide, which guaranteed a good Raman signal for the fingerprint region (1400 – 600 cm^{-1}), allowing the identification of the most common polymers found in the marine environment (the "Big six"). The novelty of this study was also that spectra were processed with an algorithm programmed to remove the Raman spectral background in a similar way for all of the spectra while keeping the analytical signal intact. Spectral extraction and identification were achieved using the SIMPLISMA algorithm model, which was modified to automate data processing. This analytical approach allowed a fast characterization and identification of a large number of microplastics particles, even when multiple polymers were combined.

MOPC10

Investigation of sorption properties and sorption kinetics of organic micropollutants onto microplastic particles

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For several years now, the pollution of aquatic ecosystems with microplastic particles, i.e. particles smaller than 1 mm, has been considered as a cause of rising concern due to their ubiquitous detection in the environment, their strong sorption capacity and their potential effects on all organisms along the food chain. Besides likely impairments of organisms due to the mere plastic material, microplastic particles are also suspected to act as vectors for contaminant transport. However, their function as pollutant carriers, which depends on material characteristics (e.g. plastic type and shape) but also on the substance properties, is still under discussion. In order to clarify this issue, sorption of six selected organic micropollutants onto different types of microplastics (particles, powders, fibres) is studied in batch experiments on equilibrium sorption and sorption kinetics. Additional sorption experiments are performed in dynamic bench-scale approaches simulating natural flow conditions in water bodies to close the gap between laboratory data and the environment. Diffusion limited sorptive uptake is mathematically described using analytical solutions for film diffusion and intraparticle diffusion which are the predominant processes in regulating sorption of hydrophobic organic contaminants. Additionally, experimental results are simulated by a numerical model using data and parameter values from analytical fits. Our results from batch experiments indicate non-linear sorption behaviour with partitioning coefficients in the same order of magnitude as given in the literature for organic pollutants and PE, e.g. derived log K_d-values for phenanthrene (Phe) are 3.76 and 4.21 L kg^{-1} for PE spherules and particles extracted from commercial facial cleaning products (peeling particles), respectively. Apparent diffusion coefficients for Phe obtained from intraparticle diffusion were in the order of 10^{-14} and 10^{-11} cm^2 s^{-1}, respectively, whereas the rate constants for film diffusion were in the range of 10^{-3} s^{-1} for peeling particles and 10^{-7} s^{-1} for spherules. Numerical model simulations resulted in water film thicknesses of 1 to 100 μm. These data indicate that the kinetic uptake of phenanthrene into spherules is mainly determined by intraparticle diffusion (possibly due to incomplete wetting) while for the peeling particles a strong influence of film diffusion at early time points and of intraparticle diffusion at later time points could be observed.

MOPC11

Investigation of adsorption behaviour of selected hydrophobic pollutants on polyethylene from different origin in different sizes

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Plastic wastes entered in the marine environment become brittle and fragmented into small pieces. These secondary microplastics (MPs) as well as primary MPs, which are manufactured to be of a microscopic size, are hardly mineralized in the environment. Thus, MPs are accumulated in the ocean and available to marine organisms. Toxic plastic additives can leach out of MPs. Persistent organic pollutants (POPs) or metals in the ocean have shown to adsorb onto and to be enriched on a surface of MPs. Microplastics in the ocean, thus, carry toxic substances to marine organisms. In order to determine the ecotoxicological effects of MPs on marine organisms, it is necessary to understand adsorption/desorption behaviour of pollutants on the surface of MPs. A series of batch experiments are in execution to study adsorption behaviour of four different PAHs on low-density polyethylene (LDPE). Specifically, we look into the influence of the polymer size and plastic additives on the adsorption behaviour of the PAHs. The LDPE used for our study varies in size (5 mm, 1.500 μm and 1.000 μm). In addition, LDPE derived from different origins (virgin pellets, pellets with additives, post-consumer product) is investigated. The progress of the adsorption is controlled using High Performance Liquid Chromatography (HPLC). The HPLC methods were optimized for four PAHs, namely naphthalene, fluoranthene, phenanthrene, benzo(a)pyrene. Freundlich adsorption isotherm is applied to derive polymer-water partitioning coefficients. As a preliminary test, adsorption of fluoranthene, a 4-ring PAH (log Kow 5,33, water solubility 0,26 mg/L) on LDPE derived from a post-consumer product was investigated. These artificially produced LDPE microplastics (0,02 g) are placed in batch reactors with fluoranthene dissolved in water with different concentrations (500, 1.000 and 2.000 μg/L). The first results showed the equilibrium was reached within 40 days and the maximum amount of fluoranthene adsorbed onto the LDPE was determined to be 14.000 μg/g-LDPE.

MOPC12

First evidence of microplastics in the African Great Lakes: Recovery from Lake Victoria Nile perch and Nile tilapia

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Microplastic contamination in the African Great Lakes is currently unreported, and compared to other regions of the world little is known about the occurrence of microplastics in African waters and their fauna. The present study was conducted in the Mwanza region of Tanzania, located on the Southern shore of Lake Victoria. The gastrointestinal tracts of locally fished Nile perch (*Lates niloticus*) and Nile tilapia (*Oreochromis niloticus*) were examined for plastics. Plastics were confirmed in 20% of fish from each species by Attenuated Total Reflectance Fourier Transform Infrared (ATR-FTIR) spectroscopy. A variety of polymer types were identified with likely sources being urban waste and consumer use. Although further research is required to fully assess the impact of plastic pollution in this region, our study is the first to report the presence of microplastics in Africa's Great lakes and within the fish species that inhabit them.

MOPC13

Chemical toxicity of virgin microplastics affects fertilization and larval development of sea urchins

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Earlier studies have suggested that virgin microplastics (MPs) used in exposure experiments could be contaminated with unknown chemicals which can leach out into the medium during exposures contributing to toxic effects. In this study the toxicity of two polymeric materials and their elutriates was tested using the sea urchin embryo test (SET). Pre-embryos of the sea urchin *Paracentrotus lividus* were exposed for 48 hours to direct, aged and leaching solutions of two types of MPs at three different concentrations: virgins microspheres of fluorescent polystyrene (PS) (10^3, 10^4 and 10^5 particles/mL) and virgin granules of high density polyethylene (HDPE) (0.005, 0.5 and 5 g/L). Aged solutions were exposed for 30 days to natural ambient conditions of air, light and temperature. Leaching solutions were obtained after filtering (0.22 µm) the solutions previously stored for 30 days under non-air and dark conditions at ambient temperature. After the exposure of pre-embryos, the percentage of larval abnormalities and larval growth were determined and compared to the control. Overall, toxic effects on the embryonic development and the larval growth of the sea urchin were found in all the treatments (direct, aged and leaching solutions) of both polymeric materials compared with the control. Furthermore, highest toxicity (% abnormalities and reduction of the larval growth > 50 %) was found after exposure to the lowest concentrations of the leaching solutions also for both types of polymeric materials. MPs ingestion was observed in the gastric cavity of the larvaes in a concentration-dependent manner, but not in a toxicity dependent manner. These findings demonstrate that virgin MPs leach unknown chemicals, e.g. additives or residual and toxic monomers, which are mainly responsible for the observed toxicity. The results of this study warrant further research and might have potentially important consequences for the interpretation of data derived from aquatic toxicity studies with (virgin) micro and nanoplastics and their extrapolation to field conditions. **Acknowledgement -** This work has been supported by IMPACTA project and by the CleanSea project, part of the European Union Seventh Framework Programme (FP7/2007-2013), under grant agreement n° 308370.

Habitat improvement in the agricultural landscape to assure the protection goal "biodiversity" (PC)

TUPC01

Development of a risk mitigation toolbox dedicated to pesticides in farmland in Europe: expected benefits for environmental protection and biodiversity and recommendations

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The registration process for Plant Protection Products (PPP) or pesticides in agriculture relies on a preliminary evaluation of the risks they may pose to human health and the environment in the farmland. Risk mitigation measures may accompany the registration in providing detailed conditions of use to reduce exposure of non-target organisms and /or residue transfer in environmental compartment to acceptable levels and thus reach the expected protection goals. These mitigation measures range from the use of special application equipment to the implementation of landscape features such as buffer strips or recovery areas, which may be recommended on product's labelling. Some of these measures are also recommended in the European Common Agriculture Policy (CAP). A two stage workshop was organized to inventory the knowledge and experience in risk mitigation measures throughout the EU. The workshop invited risk assessors and risk managers industry, academia and agronomical advisors/extension services within 21 European countries to develop a toolbox of risk mitigation measures designed for the more flexible management and use of pesticides for agricultural purposes, and thus contribute to promoting policy and practice harmonization within Europe. The workshop focused on a broad range of environmental risks: wildlife including vertebrates and invertebrates, flora and microorganisms, biodiversity as well as surface- and groundwater quality, identified as protection goals in the European regulation on pesticides. This poster will present the toolbox developed and illustrate the expected benefits of the measures recommended for the protection of wildlife in agro ecosystem.

TUPC02

INVENTORY OF POTENTIAL ECOLOGICAL FOCUS AREAS IN AGRICULTURAL LANDSCAPES IN THE CONTEXT OF THE COMMON AGRICULTURAL POLICY REFORM

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According to the EU Commission, direct payments to farmers should be linked to ecological measures (CAP Greening). One component is the establishment of ecological focus areas (EFA) on the agricultural field. The aim of this study is to identify site-specific marginal areas within agricultural sites, which could be designated as potential ecological focus areas from an agronomic and landscape-ecological perspective. These areas are mainly characterized by perennial abiotic factors that cause plant stress (reduced water capacity, soil properties etc.) These small-scale growing anomalies were inventoried based on multi-spectral satellite imagery. Two different vegetation indices (NDVI, NREVI) were calculated on the basis of high-resolution RapidEye imagery in order to assess marginal sites within predefined field blocks. Object-based hierarchical classification of site-specific areas within a field block was conducted based on a robust statistical approach by comparing sub-object vegetation indices across the entire block. Sub-object statistics of vegetation indices were compared to those of the field block in order to derive relative deviations. Four different classes were then defined using relative deviation threshold values based on overall mean field block indices values. The resulting processing method proofed to be transferable to RapidEye scenes from different acquisitions dates. The identified areas were integrated in a Cadastre of potential EFAs and can be used by decision makers and stakeholders responsible for protecting and increasing agro-biodiversity (e.g. inventory of EFA, sustainable plant protection strategies, integrated pest management and risk management).

TUPC03

Protection of wild pollinators in pesticide risk assessment and management

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Latest research on biodiversity in agricultural landscapes suggests that pollinating insects (especially wild bees and butterflies) are not sufficiently protected from the effects of plant protection products under the current risk assessment and risk management schemes. This might be the result of uncertainties in existing risk assessment practice. Currently, only honey bees are tested in risk assessment and are assumed to be representative for all bee species. Other taxa of pollinators are not incorporated, so far. Moreover, the spatial dynamics of mobile arthropods (such as wild pollinators) which move between non-target area (e.g. buffer strip) and target area (field) are not taken into account. Becoming aware of these uncertainties European Food Safety Authority (EFSA) published a scientific opinion in 2015 addressing the state of the science on risk assessment of plant protection products for non-target arthropods. The preparation of a respective EFSA guideline is planned for 2017. The research and development project Z 6 – 93 401/60 (FKZ: 3715 64 409 0), funded by the German Federal Environment Agency (Umweltbundesamt), aims to provide a basis for the revision of risk assessment and risk management schemes for the protection of wild pollinators from the effects of plant protection products. As part of this project, the current EFSA scientific opinions and draft guidance for the risk assessment of bees and terrestrial non-target arthropods will be reviewed for deficits with respect to the protection of wild pollinators. Furthermore, data on ecology, exposure and toxicological sensitivity of wild pollinators will be collected. Based on this data, ecologically tolerable thresholds will be derived and the relative sensitivity of different groups of wild pollinators in crop-specific landscape scenarios will be determined. Using this information, criteria for the risk assessment scheme on wild pollinating insects as well as recommendations for the improvement of potential risk management options will be developed. The regulatory background and the aim of the project as well as first findings are presented in this poster.

TUPC04

Ecological enhancement of agricultural land in the Upper Rhine Plain

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The project "Ecological enhancement of agricultural land in the Upper Rhine Plain" aims to find a broadly implementable way to increase the biodiversity and especially to promote and support wild pollinators and honey bees in intensively used agricultural landscapes. Therefore, a matrix of different enhancement measures like flower strips or bee banks was implemented in the intensive farmland in order to increase species richness and populations of pollinators and other farmland wildlife. A mix of annual and perennial flowering plots was used, which are manageable by the farmers in their work routine and flexibly integratable in the farmland regime. Over a time period of five years (2010 - 2014) changes in landscape structure, pollinator biodiversity and ecological parameters of arable fields were recorded. After a baseline survey in 2010, flower strips and flower fields are cultivated on 10 % of arable land within 50 ha study areas on two farms since 2011 ongoing, which are complemented by unmodified control areas of the same size. Wild bees and butterflies were sampled on flowering plots in the enhancement areas and on flower-rich structures in the control areas. Wood blocks with nesting tubes were installed to facilitate sampling of the wild bee species, and bee banks (mounds of bare soil) were created in the enhancement areas as nesting structures for ground-nesting bees. Autumn and spring sowing was carried out and the seed mixtures were adapted year by year according to the results obtained regarding an overall good variability of plant species and flowering periods, their attractiveness for pollinators, their ability to suppress undesired weeds, and their affordability numbers increased greatly on the enhancement plots. The results show that both, a variety of different flower strips/fields and a long continuity of the measures contribute to an increase of species numbers and abundance of the species. Further results regarding species composition, effects on butterflies, seed mix composition and management options are shown and discussed.To sum up, the results indicate that a broad implementation of the approach could greatly contribute to the promotion of pollinators and help to increase the biodiversity in intensive arable regions.

TUPC05

Plant Protection Products: Risk Mitigation Measures for Birds

R. Gauch; O. Daniel, Agroscope / Institute for Plant Production Sciences IPS

To ensure a safe use, measures can be disposed along with the authorisation of plant protection products to mitigate the risk to non-target organisms. Such risk mitigation measures (RMM) should be quantifiable and generally applicable to a broad range of crops. For off-field non-target organisms, the risk can easily be mitigated by applying buffer zones to surface waters or biotopes, for example. For non-target organisms occurring in the field it is somewhat more challenging to find suitable RMM. Currently applied RMM for birds in countries in and outside the European Union are presented. Further possible RMM for birds are extracted from open literature and expert questioning. The pros and cons of the RMM are evaluated and discussed regarding their potential to be used within the regulation of plant protection products.

TUPC06

Evaluation of ecological sustainability of Italian rice cultivation: identification of regulation for environmental protection and analysis of existing voluntary or mandatory agri-environment measures

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Italy is the European leading rice producer (accounting for 48% of the EU production quantities) and exporter (representing 38% of the EU rice exports). Rice cropping is almost exclusively located in the Po Valley (Piemonte and Lombardia account for 94% of the national production). In Italy rice is not only a key economic resource, but it has also a high value as environmental system. Interaction between rice cropping and the surrounding environment is very strong, by generating a real water agro-ecosystem since paddy fields enhance biodiversity and represent a core patch in agricultural landscapes. The interest of public opinion towards environmental protection and human safety is continuously growing and the European and national decision makers progressively integrated those elements in their regulatory guidance. The implemented processes descending from this regulatory system affect the agricultural practices, in the form of obligations and provisions the farmers have to follow when paddy-fields are located in protected natural areas (e.g. national and regional natural parks and reserves or sites belonging to the European Natura 2000 Network) or in the form of voluntary measures of Rural Development Plan (RDP) defined by the Common Agricultural Policy (CAP). In the presented work the authors identify these measures, which guarantee protection of environment and biodiversity as well as the promotion of environmental friendly agricultural practices, and assess their adoption by the Italian rice-growers. The diffusion of these measures and practices in terms of involved rice surface is identified with a geo-referenced analysis using GIS. As a first result it is possible to assess the strong link existing between the Italian rice cultivation and the protected natural areas. As a second step, the study highlights which are the main environmentally friendly practices and measures of the 2007-2013 Rural Development Plan (RDP) the farmers adopt, with their proportion in the main Italian Regions where rice is cultivated. Some considerations about the combination of measures and the relation of the selected measures with eventual mandatory prescriptions in protected natural areas are done. All the results are given also using cartographic methods, which enables the communication of the results in a spatial explicit way. The possibility of using this study as a methodological support to stakeholders and local territorial decision makers is also discussed.

TUPC07
Swiss agri-environmental indicators: Development of pesticide usage and aquatic risks over five years
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In Switzerland, a set of agri-environmental indicators are calculated on a yearly basis to monitor the effects of agriculture on the environment. Two indicators are directed towards pesticides (i.e. plant protection products): (1) indicator on pesticide usage and (2) indicator on aquatic risks. These national indicators can serve as important data source for better understanding the exposure of agricultural landscapes to pesticides. In this poster, we present the development of these indicators over the first five years of this agri-environmental monitoring program (2009-2013). The input data for these agri-environmental indicators are collected through a network of more than 300 reference farms. These farms annually provide their field calendars, i.e. they report for each pesticide application the applied product, crops, date and amount of applied pesticide. Based on this data, several key figures are calculated for the indicator on pesticide usage, such as number of interventions and amount of active ingredients applied per crop group. For the indicator on aquatic risks, the pesticide usage is fed into the model SYNOPS (Gutsche & Strassemeyer, 2007), which calculates the fate and the final “predicted environmental concentration” (PEC) in edge-of-field surface waters over a cropping season. The PEC value is then compared to the ecotoxicity data of each active ingredient and the risk is expressed as Exposure-Toxicity-Ratio (ETR). Risks are then aggregated over different taxonomic groups (water plants, invertebrates and vertebrates) and over entire spray-application-sequences. The risks to aquatic environments are finally displayed as aggregated values per crop group. For the indicator of pesticide usage, there are large differences between crop groups, but inter-annual variation is rather small. For the indicator of aquatic risks, there are large variations between crop groups, within crop-groups and also between years. These agri-environmental indicators provide a key information for monitoring the long-term development of the environmental impacts of the current agricultural praxis in Switzerland and help to evaluate policy instrument to reduce these impacts. In addition, the indicators can help to understand and visualize the pesticide-intensity of different crop groups within agricultural landscapes.

Oil and Gas Extraction: Ecological Effects and Science-Based Management (PC)

TUPC08
AN ECOLOGICAL APPROACH TO OIL SANDS
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The oil sands deposits of Athabasca River area, in northern Alberta (Canada), are the world’s largest oil reserves with several specific and special characteristics that make them a special case study, having both a high potential for expanding hydrocarbon extraction and also a region to assess associated cumulative environmental impacts. A key issue in the oil sands area is discrimination natural versus anthropogenic sources of oil sands related with contamination of surface and groundwater and the effects on aquatic ecosystem structure and function. The lower Athabasca River and its tributaries flow through natural bitumen deposits and consequently, it is a scientific challenge to distinguish between types and levels of hydrocarbon-associated contaminants that occur naturally in surface and groundwater from those arising from anthropogenic activities such as oil sands mining and upgrading of oil. The main objective of this study was thus to evaluate the acute and chronic ecotoxicological effects associated with the slumping of river bank material that is comprised of oil sands deposit that naturally enters the river systems through fluvial processes. For that, ecotoxicological tests were conducted using parental geological material collected from the river banks near the oil exploitation area (Alberta, Canada), in 4 different locations in 3 rivers. Samples differ each other mainly in terms of texture and proportional bitumen content due to the differences in the specific geological formations in each river basin. The solid samples (treated as soils), sediment and water-based exposure tests were carried out, using standardized ecotoxicological tests with representative terrestrial (*Folsomia candida*) and aquatic (*Daphnia magna*, *Physa acuta* and *Chironomus riparius)* organisms. Preliminary results confirmed that a suite of methodologies were needed to derive an accurate assessment of effects due to background presence of oil sands compounds. The use of both soil and aquatic organisms provided complementary knowledge on the possible ecotoxicological effects related to exposure to oil sands materials arising from bank erosion-related processes. From the results of bioassays carried out, different patterns of toxicity were observed considering the different samples’ collection areas and types of slumping material (content in bitumen), suggesting that the geological context of the area must be taken into consideration when analysing assessing ecological effects.

TUPC09
Endocrine disrupting potentials of crude petroleum related PAHs and their alkylated analogues
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Polycyclic aromatic hydrocarbons (PAHs) and alkylated PAHs are major component of crude petroleum. Spilled crude petroleum undergo weathering process and composition of alkylated PAHs could be changed. It is speculated that PAHs have endocrine disrupting potential by affecting sex hormones, and that such potential differs according to the type and alkylation status of the PAH. In this study, therefore, we evaluated the endocrine disrupting potential of five major PAHs of crude petroleum (naphthalene, fluorine, dibenzothiophene, phenanthrene and chrysene) and their alkylated analogues by MVLN-*luc* and H295R *in vitro* assay system. In MVLN-*luc* bioassay, ER binding potency of 11 PAHs were observed among total 30 tested PAHs. Among them, phenanathrene and its alkylated analogues generally showed greater potency (range of %-E2max from 1.6% to 47.3%) but the greatest ER binding potency was observed in 1-methylchrysene (101.4%). In H295R bioassay, significant increase in sex steroid hormones synthesis was observed in 20 PAHs. Although effect of alkylation on sex hormone synthesis could not be explained simply, different endocrine disrupting potential in alkylated analogues were observed. In this study, we observed steroidogenesis mediated endocrine disrupting potential of unsubstituted and alkylated PAHs rather than disruption via the ER binding pathway. This implies that steroidogenesis is important as a major pathway of endocrine disruption induced by PAHs. Therefore, steroidogenesis pathway should not be ruled out of the investigation for endocrine disruption in the oil spill site or PAH contaminated site. This work was supported by Korean Ministry of Oceans and Fisheries Project PM56951.

TUPC10
Quantifying the Role of Photochemical Processes in Aquatic Risk Assessment of Petroleum Substances
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Petroleum substances may contain polyaromatic hydrocarbons (PAHs) that interact with sunlight. These interactions can increase hazard, as a result of photo-enhanced toxicity, while reducing exposure, as a result of direct and indirect photodegradation processes. Currently, photochemical processes are not included in the PETRORISK modelling framework that has been developed and applied for conducting environmental risk assessments of petroleum substances as required

under the EU chemicals legislation (i.e. REACH). To quantitatively assess the role of light interactions on hazard and exposure assessment, available photodegradation and phototoxicity data were used to parameterize hazard and EUSES multimedia exposure models for a representative 3-ring (anthracene), two 4-ring (pyrene, fluoranthene) and 5-ring (benzo[a]pyrene) PAH. These models were then used to calculate risk quotients to aquatic life for various generic, regional exposure scenarios representing a range of sunlight exposures during winter and summer in both nutrient poor and rich natural waters. Risk quotients from these scenarios were then compared to the default case that ignored light. Results indicated that photodegradation rates were faster than biodegradation rates and of a similar magnitude for all PAHs except fluoranthene which had a ten-fold slower rate. Analysis of phototoxicity data indicated all PAHs exhibited a similar species sensitivity distribution when normalized to the combined PAH and daily ultraviolet exposure metric of micro W cm^{-2} micro g L^{-1}. Resulting risk quotients for each PAH and scenario that included sunlight were similar to or lower than the no light case since the predicted enhancement in toxicity resulting from light exposure was effectively mitigated by reduced PAH water exposure due to photodegradation. This study indicates that neglecting light interactions in generic risk assessment for PAH containing petroleum substances does not preclude effective chemical management since risk quotients are not increased.

TUPC11
Integrated Environmental Mapping and Monitoring
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Technology development, including sensors and sensor platforms, has enabled a more holistic approach to environmental mapping and monitoring. To be able to meet the environmental challenges, interdisciplinary communication and collaboration is required. As all humans share special cognitive skills in detecting and classifying objects of interest and movement with their visual system, use of imaging technology in integrated environmental monitoring have the ability of enhancing interdisciplinary communication and collaboration. To enable an extended utilisation of images in environmental monitoring, several issues still have to be further developed. This will be outlined in the presentation. However, combined use of images with other sensor data will enhance the information and facilitate improved communication and collaboration, not only between scientific disciplines but also between science and authorities and/or public in general. An interdisciplinary approach, where sensors and sensor platforms used are adjusted to purpose, can provide the flexibility needed for a knowledge driven and cost efficient environmental mapping and monitoring.

Combining exposure and effects models and data for landscape based risk assessment in a regulatory context (PC)

TUPC14
Sustainable use of Veterinary Pharmaceuticals on the territory and groundwater resources quality
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Emerging contarninants such as Veterinary Pharmaceuticals (VP) are becoming of increasing concern as consequence of their potential negative environrnental inpact. Due to their high consumption and the increasingly presence of VPs residues in water, the scientific community has become increasingly interested in investigating the effects ofthese chemicals on aquatic environments, both in groundwater and surface water systems. \nThis paper aims to address the problem of how to reach a sustainable use of VPs in the Lombardy area (North of Italy) and try to fill the gap of knowledge on how to implement appropriate risk mitigation measures on the territory to protect groundwater resources quality.\nAn exixting tool called VULPES has been used to help to plan and manage VPs use in\na sustainable way in the Lombardia Region. VULPES is a GIS-based decision support system enabling local authorities to assess the aquifer vulnerability to leaching of chemical substances by linking predictive exposure models with regional and local environmental scenarios (using GIS systems).\nScenarios will be developed using realistic characteristics of the territory (climate, soil, crop parameters, irrigation) the realistic usage of VPs and the realistic livestock practices (i.e. manure management). To achieve this objective we propose a guideline which aims to collect, organize and link information, database or tools that are already available in Lombardy Region, but that were in some way spread and scattered. In addition other relevant information has been collected from available scientific literature (predictive models, physical chemical properties ofVPs) or generated (capillary collection of information about the use of VPs).\nAnother outcome of the guideline is to raise environmental awareness of decision makers and farmers about the use of VPs in intensive livestock conditions.

TUPC15
Towards a model- and landscape-based regulatory aquatic risk assessment of pesticides: state-of-the-art and recommendations for improving fate and effect models
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Ecological modelling approaches have been recently implemented in Environmental Risk Assessment (ERA) to overcome some of the limitations of experiment-based effects assessments, namely the extrapolation across different temporal and spatial scales, and the extrapolation across different levels of biological and environmental complexity (Schmolke et al., 2010; Forbes et al., 2011). In some early examples, ecological modelling approaches were applied to answer questions regarding the possibility for propagated effects and recovery of aquatic and terrestrial populations in a spatially explicit environment context. In most of these studies, exposure assessments were performed using standard exposure scenarios and linked to population models that used ecological scenarios not necessarily matching those used in the exposure assessment. Recently, EFSA proposed to define and use environmental scenarios including ecological and exposure aspects as an umbrella under which consistent definitions of spatial and temporal boundary conditions and input parameter ranges can be collected. The idea of the definition of environmental scenarios is that the scenario selection procedures for the ecological and exposure scenarios and their parameterisation are based on common principles, allowing an integrated evaluation of fate and effect modelling results for regulatory ERA. In this presentation, we will give examples of early studies that integrate fate and effect modelling approaches at different landscape scales for aquatic (eco-)systems (Van den Brink et al., 2007; Galic et al., 2012, 2013; Focks et al., 2014, Baveco et al., 2014), and summarise a proposal for a structured approach to define ecological scenarios (Rico et al., 2015). Furthermore, we will summarise the state-of-the-art of fate models for the aquatic environment with respect to their applicability in ERA, balancing between the precision of model simulations, as needed for risk assessment, and the incorporation of large-scale systems, as required for more ecologically relevant landscape evaluations. In this presentation, the consequences of the development of ecological scenarios for the selection procedures for exposure scenarios and for the scale of the fate models to be used are identified. In addition, we will summarize the main challenges resulting from coupling fate and effect modelling approaches for pesticide registration and will propose some possible solutions as a way forward.

TUPC16
Validation of the GIS-based model SYNOPS to assess environmental risk of pesticides using four years of monitoring data in the small Lamme catchment
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The use of pesticides in agriculture causes environmental risks which must be managed carefully. On the EU level, specific Directives and National Action Plans (NAPs) have set reduction of agriculture-related pollution on the agenda. Within the German NAP, the risk indicator model SYNOPS-GIS is applied for regional risk analysis and assessment of the effects of mitigation measures. SYNOPS-GIS assesses the risk of chemical plant protection products for aquatic and terrestrial organisms. It combines data on pesticide usage with their application conditions and their inherent properties. The predicted environmental concentration of active substances is calculated on a daily basis for surface waters considering crop interception, spray drift, surface run-off and drainage. The current version of SYNOPS-GIS incorporates sub-models which are used in the European registration process, namely PRZM for the run-off assessments and VFSMOD to assess the filtering potential of buffer strips. The calculated daily environmental concentrations and the toxicity values for five aquatic reference species are used to express the risk as exposure-toxicity ratios. The risk due to multiple exposure of organisms to a variety of active substances is assessed with SYNOPS-GIS by risk addition according to the spatio-temporal concentration patterns of the active substances in the surface water. Monitoring data of pesticides in the small stream Lamme were used to validate this model approach. The upstream sub-catchment of the Lamme was part of a monitoring project conducted from 1995-1999. It is located in Lower Saxony (Germany) and includes eight fields of 109 ha. The total sub-catchment has an area of 194 ha and no settlements are located in the

catchment. The sample site is located 1.2 km downstream from the spring. The fields in the catchment were used by one farmer growing winter wheat, sugar beets and oilseed rape. All pesticide application events in the catchment were monitored. In addition weather data (precipitation, temperature) were measured and data on landscape structures (e.g. buffer zones, field margins), soil and slope were derived from detailed GIS datasets. Of the37 active substances applied over the time period of four years, 14 were detected in the weekly composite samples. These monitoring results are correlated with predicted concentrations of SYNOPS-GIS and the results are discussed.

TUPC17

Development and application of a modular landscape-scale vole model for pesticide risk assessment combining the models Xplicit and Polaris

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Recently, the potential of conducting landscape-level risk assessments has been discussed to predict potential effects of pesticides on non-target organisms at landscape scales (e.g. EFSA 2015a and b). This requires on one hand (i) to develop landscape model approaches, and on the other hand (ii) to develop scenarios fulfilling requirements of regulatory risk assessment and management. Landscape-scale modelling comes with a range of disciplines, ranging from modelling of environmental conditions, data and knowledge on land and farm management, exposure and efate modelling, ecological modelling, and in particular risk characterisation according to protection goals. All of these fields are in dynamic development and require specific knowledge. On this background, and due to the fact that a variety of very specific models often exist, we adapted a modular approach which allows combining individual model components using standardised interfaces. This not only allows to flexibly combine of models and to increase the level of complexity step by step in the risk assessment, but it also helps risk assessors to efficiently evaluate risk assessments, because once modules are validated they can be reused without the need of re-validation. In this respect, this approach also facilitates harmonisation. In a first implementation of this architecture we combine modules from a landscape-scale model (Xplicit; environmental conditions, land use/cover, PPP use, exposure, efate) with a vole model (Polaris), as well as modules for risk analysis. Due to its openness, it is straightforward to introduce e.g., new landscape data. This approach is used to analyse relationships between landscape characteristics (composition, structure, management, scales) and vole population dynamics, with and without pesticide use, in order to identify realistic worst-case scenarios for risk assessment (EFSA 2015c, Wang and Luttik 2013). In this poster we present details on the modular landscape modelling concept and its implementation for a vole risk assessment. We show that the model can flexibly be used at different scales and different risk assessment tiers (complexity level). In addition, we illustrate how the landscape-scale modelling approach can be used to identify which factors determine the level of susceptibility of populations.

TUPC18

Comparing observed and predicted honeybee nectar-foraging at landscape level

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We applied a landscape-level nectar foraging model using the spatial settings associated with a field experiment on honeybee foraging. The model calculates foraging behaviour based on energy balance of honeybee foragers for a single day in complex landscape mosaics. The hive population is assumed to select the resource patch with highest net energetic efficiency and to have perfect knowledge of its environment. By using real-world GIS data, including semi-natural elements such as field margins and buffer strips, present within a radius of several kilometres, the model can quantify foraging costs and yields in terms of energy for the specific field situation, and based on these calculations the amounts of nectar from the different fields and other nectar sources in the neighbourhood arriving at a bee hive. In the experiment a 1 ha flowering field of oilseed rape, was sprayed with a non-toxic tracer. After application, during the remainder of the day, bees were collected approximately every hour in the sprayed field and at the entrance of the two beehives situated next to the field. Tracer residues differed between field and hive collected samples hinting towards dilution at the hive or the presence of other yet unknown factors. We used the model to test several plausible explanations for the observed experimental results. In particular, the hypotheses were tested that either the presence of attractive alternative nectar sources in the wider landscape could have resulted in less than expected numbers of foragers visiting the crops, or that a large fraction of what were considered foragers were in fact apprentices, learning to practice their foraging skills. Vice versa, the experimental results helped to perform a reality check for the foraging model by comparing observed to simulated nectar concentrations. In particular, the assumption was tested that at any time, only a single, optimal, resource would be exploited.

TUPC19

A bug's eye view - quantifying pesticide heterogeneity at micro-scales needed to model non-target arthropod behaviour in orchards

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Pesticide exposure within fruit orchards has been widely studied, but rarely at more than one spatial scale; in addition it is usually undertaken in the context of human exposure. Here we take a different approach. We aim to quantify pesticide exposure at scales relevant to individual non-target arthropod behaviour. Such species are included in the regulatory risk assessment of pesticides, and may provide important ecosystem services. We assessed five spatial scales during a spray application of the fungicide penconazole in an apple orchard in the UK in August 2015 and used two residue analysis methods: pesticide extraction from apple leaves and analysis via GC-MS; and computer-based image analysis of pesticide deposition on water sensitive paper. We report residue levels focusing on the spatial scales most relevant to two non-target arthropod species widely used in both biocontrol and pesticide risk assessment: the green lacewing *Chrysoperla carnea*, and the predatory mite *Typhlodromus pyri*. In addition we evaluate the potential for water sensitive paper to be used in residue studies instead of cost- and time-intensive analytical techniques. Our results feed into a wider project to produce a model that predicts the population-level consequences of lethal and sub-lethal effects of pesticide exposure, including potential avoidance at the micro-scale, on the two non-target arthropod species.

Communicating research findings and uncertainties: Strategies, tools, and new platforms for environmental sciences (PC)

WEPC01

Contrasting public perception of pollution in a small European city and an East Asian megacity - the case of York versus Seoul

L.H. Youds, University of York / Environment

Around the world, a shift towards urbanisation is being realised. Currently, over 50% of the world's population live in cities and this is expected to increase to 66% by 2020. With higher rates of urbanisation come greater levels of pollution. In cities, this encompasses a diverse range of issues under noise, air, soil and water pollution themes. Pollution in cities is an important problem due to the effect that the variety of pollutants may have on human health and wellbeing and the health of the natural environment. The rate and extent of urbanisation varies considerably around the world, thus the extent and composition of pollution in different cities also display great variety. Urbanisation in Europe stands at 73.6%, whilst in some East Asian countries, such as South Korea and Japan, urbanisation stands at higher rates of 82.5 and 91.3%, respectively. Although the extent of urbanisation in both regions is not wildly variant, the above figures hide more interesting information on the significantly different historical development of European versus East Asian cities and the impact that these development trajectories have had on culture, public policy, economic structure, the environmental, social and physical characteristics of cities, and vice versa. This poster presents the findings of a comparative study of public opinion on pollution in a small European city (York, UK) and an East Asian megacity (Seoul, South Korea). The findings are presented within the context current pollution- and environment-related attitudes of citizens within each city and will include measures of: citizen engagement in the use of innovative technology and social media for monitoring personal exposure to pollutants; participation of citizens in public policy decision-making; and behavioural trends in terms of limiting personal exposure to pollutants and reducing environmental footprints. In addition, the historical developmental trajectory of each city will be discussed and the possible impact that culture, economic development, rate of urbanisation over time and governance may have on the varying attitudes and opinions of each city's public. The findings of this study have significant implications for beginning to understand how different cities digest, value and respond to information on pollution problems in substantially different contexts.

WEPC02

Uncertainty avoidance in environmental risk assessment - current guidance and future challenges

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Uncertainty estimations are inherently built into any scientific process, not excluding environmental risk assessment (ERA). Although usually hidden behind safety factors and policy decisions, in recent years, with the development of new assessment methods, uncertainty has become more explicit. Despite this, most people, including risk assessors and managers, avoid uncertainty and often display uncertainty-averse behaviours when making decisions. Uncertainty avoidance describes the level of tolerance of uncertainty and ambiguity, where choices are biased towards assigning greater weight to information about uncertainty in comparison to the rest of available data. As to date, we know little about the social and psychological factors contributing to that phenomenon. The aim of our study is to systematically analyse different ways of addressing uncertainty in ERA, using existing (or proposed) guidance and legal frameworks for ERA, focusing especially on uncertainty perception and sources of uncertainty beyond statistical estimations. We identified gaps, challenges and suggested ways to incorporate, so far overlooked, psychological factors, especially uncertainty avoidance, into risk management. Our preliminary results show that both perceived uncertainty and uncertainty avoidance have been so far overlooked in ERA regulations. The most general decision-making guidance, the precautionary principle refers to "unacceptable risks" and scientific uncertainty, but leaves them in the domain of political responsibility. Currently, only ESFA's draft guidance document explicitly acknowledges perceived uncertainty in ERA. Whereas uncertainty analysis is a well-established part of ERA guidance documents, a systematic, empirical analysis of perceived uncertainty in ERA and especially of uncertainty avoidance is lacking. Existing probabilistic models incorporating uncertainty in ERA do not take into account individual or social variables. Still, the awareness of these kinds of influences on decision-making processes is growing. To keep ERA ready for emerging risks, it is not enough to develop new assessment methods and more accurate predictions. Underlying reasons for uncertainty avoidance need to be addressed to facilitate informed risk communication.

WEPC03

Accounting for Environmental Recovery in Risk Assessment and Management

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The Energy Institute (London, UK) has developed a guide for risk assessors to determine the duration of environmental recovery following major accidents to the environment. The guide is designed to support site safety reporting for the UK Control of Major Accident Hazard (COMAH) Regulations, which implements the SEVESO Directive (2012/18/EU). The information gathered on environmental recovery may also have wider uses for predicting recovery in damage and compensatory assessments (Environmental Liability Directive 2004/35/EC) and evaluating risks as part of contaminated site assessments. The guide is currently undergoing final sign off for publication in 2016. The aim is to provide easy-to-use guidance for defining environmental recovery based on major accident scenarios for releases of SEVESO substances, including petroleum based products. The guide provides a framework for the selection of appropriate recovery criteria for a range of temperate habitats using typical release scenarios for hazardous substances. The primary audience for the guide will be managers and their advisors responsible for the preparation of Site Safety Reports by establishment of risk screening for refineries and chemical production facilities together with storage terminals. The approach includes a review of published literature on habitat and species recovery from accidental spills. In parallel, the physico-chemical properties, environmental fate and ecotoxicity of chemicals captured by COMAH/SEVESO III were evaluated to identify persistent, bioaccumulative and toxic (PBT) substances with the potential for longer-term impacts/delayed recovery. Reliable studies are used to compile recovery trajectories resulting in recovery durations for a range of habitats. These are presented in a step-wise framework for assessors to identify an appropriate recovery duration based on their chemical(s) of interest and types of habitats on and around their sites. Taking account of environmental recovery in an assessment is often neglected, but understanding the consequences of an accidental release is important to valuing the scale and severity of an impact. Recovery information could be used to direct appropriate management actions (as in COMAH/SEVESO) and avoid under- or overly-conservative compensatory actions in dealing with environmental damage and liability.

WEPC04

ECHA and communicating scientific content

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ECHA is an Agency of the European Union with the responsibility to manage four pieces of European chemicals legislation: REACH, CLP, Biocidal Products Regulation (BPR) and PIC. The objective of all of them is to ensure a high level of protection of human health and the environment, as well as to enhance competitiveness and innovation of industry. The Agency needs to raise awareness and support duty holders – chemicals companies - in complying with the legislation. ECHA also needs to use the results of the legislative processes to promote the safe use of chemicals among downstream users, workers, consumers and other general audiences. This task requires content which explains the scientific data in an easily understandable way and in the relevant context. In order to achieve its communication objectives, ECHA needs to be realistic, strategic and consistent in its communication. The main communication principles are the following: Accessible. Communication vehicles are easily accessible, transparent and use understandable language. Strategic. ECHA focuses on defined priority topics and targets the key audiences. Reliable. ECHA's communication is accurate and predictable. Integrated. ECHA uses a variety of communication channels and vehicles to optimise the outreach and targeted messaging. EU level. ECHA's messages are targeted for audience segments but not for individual countries. Work with multipliers. ECHA engages EU level multipliers and member state competent authorities to enable targeted messaging and efficient outreach. Multilingual. Information intended for SMEs and general audiences is available in 23 EU languages. At SETAC, ECHA could present how it communicates scientific content to general public, covering different channels and modifying the content accordingly. This includes the use of ECHA's social media channels, audio-visual products and its dedicated web page to the general public, "Chemicals in our life". Regarding content, ECHA communicates regulatory matters but also content related to recent scientific developments, as nanomaterials, endocrine disrupters or alternatives to animal testing. ECHA also communicates directly with scientists, for example through organising topical scientific workshops and participating at events (SETAC, Eurotox). In addition, ECHA could present how it coordinated a risk communication network with the Member States and produced a guidance document on risk communication.

WEPC05

Post-modern times - The EcotoxBlog

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Nowadays, communicating research results to 'outsiders' has become an essential part of academic activities with diverse goals that need to be met. These include raising public awareness about environmental issues, communicating science directly to the public or attracting students to universities. Taking advantage of social media can be an effective way to achieve these aims. As a part of a larger public outreach campaign of our university, we thus started the EcotoxBlog in May 2015 not only to improve public visibility of our Ecotoxicology-related research and our Master's program in Ecotoxicology, but also to foster internal communication and increase awareness of research-related activities. Since then, we regularly post summaries of our scientific work, written in non-technical language that is clear and comprehensible to a wide and potentially non-scientific audience. The summaries are comprised of the latest publications, novel projects, and updates about ongoing research in the various working groups. Moreover, we use the blog as a pin board to inform our students about all news related to their studies and future careers (e.g., job advertisements). The students welcome this new tool and we have recruited two student bloggers, who regularly write well-received contributions about their and their fellow students' experiences during the Master's course (e.g., lab courses or internships) as well as their first contacts with the scientific community (e.g., first scientific meetings). This blog is also an open platform to interact with colleagues and the larger public and anyone is free to contribute topically related tidbits of information. To draw the attention of irregular visitors to the EcotoxBlog, we also operate Facebook and Twitter accounts, where we advertise each blog post. Moreover, within the university, regular email newsletters inform staff and students about the latest posts, and everyone can subscribe to an RSS feed to receive all the news. Although only running for half a year now and still optimizing our posting strategies, the EcotoxBlog has proven to be an effective tool to communicate our research and the content of our study program to a diverse audience: lots of positive feedback and several hundred users per month from all over the world show that we are on the right track. So join us at http://www.master-ecotoxicology.de/ecotox-blog/.

WEPC06

Gamed-based tools as media to transmit freshwater ecology concepts

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There is an increasing expectation of people to be aware of the environmental issues; however, expert knowledge is often required to understand most of them. Thus, games could turn out to be a good medium for scientific vulgarization. Gamification has already been proposed as a tool for the propagation of scientific thinking. In this context, our project aims at developing game-based tools to transmit the basic concepts of freshwater ecology. We chose to focus on a classical board game and on a computer-based game because they are complementary in the targeted audience and the possibilities offered (interactions between players and the system dynamics). While the board game is inspired by past experiences of player, we propose to implement an agent-based model (ABM) for the computer version and to couple its dynamics with gaming actions. ABM have already been widely used in ecology, therefore, we selected a trophic chain dynamic model that captures species behavioral rules and spatially heterogeneous environment. We chose to represent the ecosystem from a fish perspective and to base both games on the same general rules. The player objective is to reach a given number of adults and juveniles that guarantee the stability of the fish population in a lake. For this purpose, each player has to find resources and to use them for different actions (e.g. reproduction). The perturbations of the ecosystem are illustrated by "events" designed to reflect abiotic (e.g. water temperature) and biotic (e.g. parasites, human) stressors. The current versions of the games include four players (fish species): roach, pumpkinseed, zander, and bleak. Our idea is to maximize the interactions between players (predation and competition) and to illustrate various feeding and reproduction strategies. A prototype of each game is currently available for testing and refinements are expected while testing the games. In a short term, the next versions will integrate the aesthetic design (edge of a lake) and refined processes parameters (model calibration). In a long term, we aim to develop an online version of the computer game and to use crowdfunding platforms to diffuse the board game. The very first objective of our games remains to be entertaining, keeping in mind that the ludic rather than educative aspects are central in such game-based media. If players forget that it is about ecology, it would mean that the underlying scientific concepts are understood, exactly what we want.

WEPC07

Heavy metal assessment in agricultural soils contributes to a sustainable information of the public regarding soil quality in a developing country

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Cuba is mainly an agricultural country and healthy food production depends on the quality of the soils. Several studies in Cuba propose reference values of heavy metals (HMs) indicating the quality of different soil types with little human activity. Nevertheless, systematic monitoring studies of HMs in soils of Cuba are scarce. Heavy metals are prone to be taken up by plants, which may affect food safety. To inform the stakeholders (consumers, farmers, government) about the quality of Cuban soils, a sound monitoring of contaminant exposure forms the base for an appropriate and adequate risk assessment, and corresponding communication with stakeholders. Our project Soil-Q, jointly carried out by the Cuban agricultural research institute CENSA and the Swiss Agroscope ISS aims to establish a monitoring network to assess the contamination status of Cuban soils. So, 39 sample locations with agricultural and rural character in the province of Mayabeque were monitored according to the rigorous protocol of the Swiss soil monitoring network. The mean concentrations of HMs (cadmium 2.6, chromium 63, copper 64, nickel 48, lead 16, zinc 55, and mercury 0.1 mg/kg_{soil}) measured in these sites do not exceed the intervention thresholds according to the regulations of Germany and Switzerland. However, the values higher than the median appeared in agricultural soils. Additionally, Soil-Q is to raise perception and awareness of the stakeholders. A film in the Cuban TV was shown and different workshops for representatives of related research and governmental institutes were held. The message was to inform about Soil-Q and that first results do not show a risk for humans and the environment. It is crucial to inform the general public as well as decision makers that this is only the beginning of a monitoring process that should extend over the whole country and over decades because soil is an important archive about human activities. Especially in a country where future changes are very likely, a long-term monitoring catching industrial and other human activities is of uttermost importance for the welfare of humans and the environment. It is a big challenge to maintain an information flow with projects that are finite. Public perception towards the quality of the environment, especially as there seems to be not an acute problem is very difficult to keep active. We hope therefore to learn more about this subject at this session by communicating and networking with other experts.

Endocrine Disruptors: Exposure, Hazard & Risk Assessment (PC)

WEPC08

When are risk assessments of endocrine disrupters scientifically sound and secure?

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The poster summarises the consensus that resulted from the SETAC Pellston™ Workshop: Environmental Hazard and Risk Assessment Approaches for Endocrine-Active Substances (EHRA), held at Pensacola on 31 January – 5 February 2016. The primary aim of the workshop was to provide objective advice, based on the current level of scientific understanding, to enable regulators/policy makers to make considered, informed decisions on whether to select a hazard- or a risk-based approach for a given endocrine-disrupting substance (EDS) under review. The workshop additionally considered recent developments in the identification of EDSs. All guidance developed was based on experience obtained from working on case histories of six data-rich endocrine-active substances (EAS) - ethinylestradiol, perchlorate, propiconazole, trenbolone, tributyltin and vinclozolin. This is a controversial subject because it is claimed by some that environmentally safe concentrations/doses of (certain?) EDSs cannot be established due to certain inherent properties *e.g.* unusual concentration/dose response curves; long-term effects resulting from short-term critical windows of exposure; difficulties of extrapolating to effects in untested life-stages and species *etc*. The implication is that such substances should be regulated solely on the basis of their intrinsic hazards. This poster summarises information on current best practice in the environmental hazard and risk evaluation of EDSs which was developed using a cross-section of international expertise on this issue. The core advice to regulators and policy-makers faced with a confirmed EDS is to ask a structured series of questions, as follows: Is exposure prediction reliable? Have relevant taxa and life stages been tested? Are tests adequate to detect delayed effects? Are tests likely to have missed adverse effects at concentrations below those tested? These questions are underpinned by detailed guidance on how they can be addressed, and at each stage, users are invited to fill in data-gaps as appropriate. Only if the required additional testing is not deemed feasible should regulation on the basis of hazard alone be considered until such time as suitable methodology becomes available. The overall conclusion of the EHRA SETAC Pellston Workshop™ was that if the questions above are adequately addressed, then conducting environmental risk assessment of EDSs is scientifically sound.

WEPC09

Uncertainties in biological responses that influence hazard or risk approaches to the regulation of endocrine active substances

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Endocrine Disrupting Chemicals (EDCs) may have delayed or transgenerational effects and display non-monotonic dose response relationships (NMDRs) that require careful consideration when determining environmental hazards. The case studies evaluated for the SETAC Pellston Workshop™: Environmental Hazard and Risk Assessment Approaches for Endocrine-Active Chemicals and other key examples from the literature are discussed. EDCs can have specific and profound effects when exposure occurs over sensitive windows of the lifecycle (developmental, reproductive). This may induce delayed effects where the adverse effect is manifest at different lifestage(s). This underscores the need for testing in appropriate (sensitive) lifestages and full lifecycle designs that capture adverse effects wherever they occur in the lifecycle. Such tests are available in the toolbox and should be employed to derive endpoints that cover all life stage sensitivities. Similarly, the potential for effects to become manifest in subsequent generations (transgenerational) has also been raised as a potential issue in the derivation of appropriate endpoints for EDCs. However, the evidence for such effects as a general issue is limited. Indeed this is reflected in the design of new higher tier tests to assess EDCs developed under the auspices of the OECD and US-EPA by the move to extended one-generation designs and away from multi-generational studies for fish and mammals. The occurrence of non-monotonic dose or concentration response relationships is also considered a limiting factor for reliable risk assessment of EDCs. Substantial data reviews are underway to inform on their occurrence and relevance. However, evidence to date indicates they are more prevalent in *in vitro* and *in vivo* mechanistic data, not often translating to adverse apical endpoints that would be employed in risk assessment. A proposal of how to evaluate NMDRs in the context of endocrine hazard and risk assessment procedures is presented. If careful consideration of delayed, transgenerational and NMDR effects is made, it is feasible to assess environmental endocrine hazards and derive robust endpoints for risk assessment procedures ensuring a high level of environmental protection.

WEPC10

Assessing the Effects of Endocrine-active Substances on Populations

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For ecotoxicological risk assessment, endocrine disrupters require: 1) mechanistic information to demonstrate an endocrine mode-of-action (MoA), and 2) a plausible linkage between this MoA and an adverse effect that is relevant at the population level (WHO IPCS, 2002). In establishing MoA, data on additional endpoints (subcellular-through-organ levels) are collected for different taxa, but their link to adverse population level effects is often unclear. Case studies of endocrine-active substances (EAS) (tributyltin, ethinyl estradiol, perchlorate, trenbolone, and vinclozolin) were used to evaluate the population relevance of toxicity endpoints for use in hazard and risk assessment. The data for various taxa (invertebrate, fish, amphibian, avian, and mammalian) were examined using the OECD Conceptual Framework for Testing and Assessment of Endocrine

Disrupters to determine the relevance to adverse, population level effects. For some EAS like tributyltin, population relevance of mollusk imposex is well established. In fish, altered secondary sex characteristics by vinclozolin did not affect reproduction in a medaka extended one-generation study, and were judged to be not relevant for fish populations. Higher concentrations of vinclozolin decreased reproduction in fish, which is population relevant. The amphibian metamorphosis assay can detect altered stage distribution of tadpoles, which is predictive of increased time to complete metamorphosis, and might result in adverse effects for amphibian populations. In birds, population-relevant endpoints include egg production, hatchability, embryo viability, growth and egg condition. Decreased tibia-femur length (i.e., growth) in developing birds was seen with the thyroid-active compound, perchlorate. In mammals, anti-androgens like vinclozolin can affect reproductive development (e.g., decreased anogenital distance, nipple retention), but the population relevance of these endpoints is uncertain. In longer-term studies, vinclozolin reduced male rat fertility and successful mating, effects that are population relevant. Recovery processes also are important when evaluating the adverse effects of EAS on wildlife populations. Lastly, new methodologies (e.g., adverse outcome pathways - AOP and modelling) will aid in our understanding of endocrine perturbations and adverse population level effects once our knowledge on key event relationships improves.

WEPC11

Current limitations and a path forward to improve testing for the environmental assessment of endocrine active substances

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To assess the hazards and risks of possible endocrine active chemicals (EACs) there is a need for robust, validated test methods that detect perturbation of endocrine pathways of concern and provide insights reliable information as to assess to potential adverse effects on apical endpoints. One issue of significant concern to for current EACS screening/testing programs involves resources in regard such as to cost, time, trained personnel, and animal use. This is especially problematic when considering the number of chemicals that some regulatory authorities need to assess. One way to address this challenge is to prioritize chemicals for possible *in vivo* testing using *in vitro* high throughput (HTP) assays focused on a suite of endocrine molecular initiating events (MIEs) of concern. Additional challenges associated with the design and conduct of *in vivo* EAC screening and testing include selection of appropriate species (*i.e*., sensitive and amenable to laboratory testing), endpoints and life-stages. A component of this involves experience gained using existing tests to determine, for example, those that have been shown to be exceptionally sensitive to perturbation of a given MIE of concern. However, there is the opportunity for strategic use of HTP data and/or early screening level information to help guide the selection of existing assays that can further evaluate a given EAC modality. Further challenges for EAC screening and testing involve guidance and optimization in several areas, such as concentration setting, statistical power to detect biologically significant adverse effects, delivery and analytical measurement of test substances, availability of technical expertise, and study interpretation, including linking mechanistic and apical effects. Some of these areas can be addressed by the lessons learned and best practices developed through recent experiences conducting EAC screening/testing. Additionally, the collective assessment of EAC screening and testing data (*e.g*. compilation of historical control data) can be leveraged to refine test designs and performance criteria to maximize the power and utility of EAC screening/testing. Finally, a number of recommendations are provided for longer term research to address areas of uncertainty, including identifying potentially sensitive species for which test methods currently do not exist (*e.g*., invertebrates) and key endocrine pathways in addition to estrogen, androgen and thyroid signalling.

WEPC12

Challenges in Assigning Endocrine Specific Modes of Action: Recommendations for Researchers and Regulators

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As regulatory programs focus on evaluating substances for their endocrine disrupting properties, there is a need for careful study design and data interpretation to distinguish between endocrine versus non-endocrine specific responses. This is particularly important where specific criteria are under development to identify endocrine disrupting properties to enable hazard-based regulation. Irrespective of the regulatory process, most jurisdictions use the WHO IPCS definition of an endocrine disruptor (ED), requiring that a substance is demonstrated to cause a change in endocrine function that consequently leads to an adverse effect in an intact organism. Such a definition is broad, and might capture mechanisms that in general can be both, substance specific and substance-non-specific endocrine responses. For instance, stress is a non-specific, neuro-endocrine response that can lead to adverse outcomes. In addition, non-endocrine toxic mechanisms (e.g. hepatotoxicity, acetylcholinesterase inhibition) may operate secondarily or in parallel to impact the endocrine system and apical endpoints downstream. Furthermore, endocrine responses may be adaptive in nature and designed to maintain homeostasis rather than inducing an irreversible adverse effect. The likelihood of indirect effects is increased in (eco)toxicological studies requiring the use of maximum tolerated dose levels, which must produce some adverse effect. The misidentification of indirect effects as truly ED has serious consequences in terms of triggering animal and resource intensive testing and potentially severe regulatory consequences. A review, based on 6 case study substances, was conducted to evaluate scenarios that could complicate the assessment of whether or not a substance is an endocrine disruptor. In order to achieve this objective, a weight of evidence approach was used to evaluate available data for the case-study substances. In this approach, the weight of evidence was based on biological plausibility, empirical support, and essentiality of key events in adverse outcome pathways. A process is recommended where indicative (endocrine specific and non-endocrine specific) and apical endpoints can be evaluated to investigate whether an endocrine mode of action can be conclusively assigned to the effects observed for a given substance. Two examples are discussed each with varying degrees of endocrine mode action specificity.

WEPC13

Evaluating the Credibility of Histopathology Data in Environmental Endocrine Toxicity Studies

J.C. Wolf, Experimental Pathology Labs., Inc.; G. Maack, Federal Environment Agency / Ecotoxicological Assessment

Agencies responsible for environmental protection are tasked with developing regulatory guidance that is based on the best available scientific evidence. Histopathology is a common endpoint in toxicological bioassays, and advantages of this endpoint for ecotoxicological research include: (i) reasonably high degrees of sensitivity and specificity relative to other endpoints; (ii) the ability to identify and/or confirm novel or unanticipated treatment effects that may not be detected by other means; (iii) the ability to reveal the presence of subclinical (and potentially confounding) background disease; (iv) the ability to link effects obtained from molecular or *in vitro* experiments to more apical population-relevant endpoints; and (v) in some cases, the provision of key mechanistic information pertaining to toxicity. However, because of the subjective nature of histopathology, and the advanced level of specialized training required for the effective utilization of this endpoint, the reliability of histopathology data can be inconsistent. Consequently, mechanisms for evaluating such data on a case-by-case basis are needed. The purposes of this report are to describe methodology that can be used to evaluate the credibility of histopathology findings in published articles, and discuss the results of such assessments as applied to real-world data gathered from the scientific literature. The ultimate goals of this work are to draw attention to reliability issues that can affect the histopathology endpoint, provide recommendations to improve the quality of this endpoint, and suggest an approach for the expeditious and judicious use of histopathology data in weight of evidence determinations required for hazard and/or risk assessment. This exercise was conducted initially as part of a SETAC-Pellston Workshop™ entitled "Environmental Hazard and Risk Assessment Approaches for Endocrine-Active Chemicals (EHRA): Developing Technical Guidance Based on Case Studies to Support Decision Making" that was held in Pensacola, Florida, USA, from January 31st to December 5th, 2016.

Passive sampling of organic micropollutants and toxicity assessment: opportunities, challenges and innovations (PC)

WEPC14

A comparison between passive samplers and bioassays to monitor the release of produced water from the oil and gas industry in the water column

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In 2012, the total production of oil and gas in Norway represented 226 million cubic meters of oil equivalent. Produced water (PW) represents the largest volume waste stream in oil and gas production operations from most offshore platforms. In 2012, around 130 million cubic meters of PW was discharged by the

Norwegian offshore oil and gas industry. PW includes both formation water (seawater or freshwater trapped with oil and gas in a geological reservoir) and injected water (seawater, freshwater and brine water as well as added chemicals that are injected to enhance recovery of oil and gas, and operational safety) and as such contains components such as dispersed oil, aromatic hydrocarbons, alkylphenols, organic acids, heavy metals, radioactive materials and inorganic salts. The exploration of oil and gas takes place offshore in sensitive coastal environments and as such stringent monitoring regulation must be followed. The currently used water column monitoring strategy includes the use of passive samplers and caged organisms to monitor the effects of releases of PW. Passive samplers and caged organisms must be placed on all monitoring stations and passive samplers should be used to monitor dispersal of discharge to the environment. This work investigated the relationship between monitoring results obtained via the use of passive samplers and organisms using a real PW sample as a case study. Four passive samplers (polyethylene, polyoxymethylene, polyethersulfone and polydimethylsiloxane) were calibrated for PAHs and alkylated PAHs by determining respective passive sampler-water partitioning coefficients. A small field deployment in the Oslo fjord was carried out to investigate ease of deployment. Polyethylene and polydimethylsiloxane were identified as the most optimal passive samplers monitoring chemicals in PW. Polyethersulfone did not function as an equilibrium passive sampler, as expected, and was very difficult to deploy as a single membrane in the field. A battery of bioassays were then carried out in using the PW sample and also extracts from the selected passive samplers that had been exposed to the PW sample. In addition the PW was diluted to simulate increased distance from the release point and the bioassays were carried out again. The correlation between concentrations determined with passive samplers and the ecotoxicological response to the organisms will be investigated.

WEPC15
Assessing the toxic potential of river water by combining passive sampling with bioassays
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Bioanalytical tools can provide important information on the toxic potential of complex chemical mixtures of environmental samples and are, therefore, complementary to chemical analysis. The combination of passive sampling with bioassays is a promising approach for assessing water quality as 1) passive samplers accumulate a broad spectrum of chemicals while excluding most of the matrix and 2) chemicals with the same mode of toxic action are detected in parallel. Up to now, the majority of studies investigated solvent extracts of polar organic integrative chemical samplers with the yeast estrogen screen (YES) to assess contamination of aquatic environments with chemicals causing estrogen-like activity[1]. To study the toxic potential of river water, silicone-based samplers were exposed in rivers for five weeks, extracted with n-heptane in a Soxhlet-apparatus and then extracts were tested in bioassays. In preliminary experiments, extracts from passive sampler that had been exposed in the rivers Rhine, Danube, Elbe, Saale and Saar were tested in bioassays measuring mutagenic (Ames fluctuation assay) and endocrine activity (YES). Thereby, samples from the river Saar showed high mutagenic potential in the Ames fluctuation assay, in particular when using the strain YG 1041 that is sensitive to nitroaromatic compounds and, after metabolic activation by S9-mix to aromatic amines. Therefore, a more comprehensive monitoring program was conducted along the river Saar in 2015, whereby passive samplers were exposed at twelve sampling sites in the river Saar, its main tributaries, and in the effluent of a wastewater treatment plant. All samples showed mutagenic potential with highest effects in samples from sites located in the Saarland conurbation, an important old-industrialised region in Germany, as well as in samples from the main tributaries. In most cases, mutagenic effects were detected after metabolic activation with S9 mix, whereas no or only minor mutagenic potential were found without metabolic activation. This indicates that the mutagenic potential might primarily result from aromatic amines and not from nitroaromatic compounds.\n
\n [1] Jahnke, A. et al. (accepted): Combining passive sampling with bioanalytical profiling of mixtures of pollutants in the aquatic environment. *In*: *In vitro* Environmental Toxicology: Concepts, Application and Assessment, Editors: Reifferscheid, G. and Buchinger, S., Series: Advances in Biochemical Engineering/Biotechnology, Springer \n

WEPC16
Screening methods for fate and toxicity of produced waters
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There is a need to improve the risk assessment of offshore produced water (PW) discharges and find a rapid and cost effective alternative to logistically challenging WET testing. A simple battery of monitoring techniques, which closely mirrors the OSPAR Risk Based Approach (RBA), has been trialled in a wide range of facilities. This study has applied solid phase micro-extraction coupled with gas chromatography (SPME-GC), a biomimetic technique, and the *in-vitro* Microtox assay (*Vibrio fischeri* bioluminescence assay) for the purpose of produced water toxicity assessment. These techniques will ultimately form part of a toolbox for early tier routine screening of produced water effluents. A collection programme of PW samples was coordinated from a number of offshore PW waste streams. Aliquots were simultaneously tested using Microtox to describe the toxicity of both known and unknown components, and SPME-GC to determine the soluble, and thus bioavailable, hydrocarbon concentration. Components desorbed from the fibre were quantified as total dissolved petroleum hydrocarbon concentration, by calibration against hydrocarbon standards. Toxicity (assessed using Microtox) increases in line with dissolved hydrocarbons determined by SPME-GC suggesting hydrocarbon contamination to be a major contributor to toxicity. Biomimetic techniques such as SPME will also reflect the potential of a substance to bioaccumulate and thus provides more ecologically relevant data. Initial BCF testing has demonstrated bioaccumulation potential of PW to be of low concern. In due course, Microtox and SPME-GC screening will assist with other screens in PBT determination, fulfilling Step 2 of the RBA by providing toxicity and bioaccumulation data.

WEPC17
Mobile passive sampling as a tool for toxicological profiling
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Many components of complex organic pollutant mixtures in waters are often present at very low levels, posing a challenge for current analytical methods. Passive sampling is one of the perspective methods that could address this problem. In our work, we used a mobile "active" passive sampling system with two types of sorbent (EMPORE discs, silicone rubbers), which preconcentrates a wide spectrum of polar and non-polar organic pollutants from the water column. Eight Danube river stretches were sampled and spatial distribution profiles were described for a comprehensive range of organic pollutants including pesticides, pharmaceuticals and industrial chemicals. Sampling rate and the original concentrations of the pollutants in water were estimated for pollutants with different physicochemical properties by a set of reference compounds. The employed mode of passive sampling with an active exchange of water in the sampling chamber proved to be about five-times more effective than static exposure and in relatively short sampling times allowed quantification of chemicals in pg.L^{-1} levels. Beside chemical analyses, samples were characterized with a battery of *in vitro* bioassays sensitive to chemicals with selected modes of action. Toxic potentials for causing endocrine disruption (anti/estrogenic, anti/androgenic), dioxin-like activity, pregnane X receptor (PXR)-mediated activity and oxidative stress-associated response showed spatial variation along the river. Polarity of chemicals played an important role, while AhR-mediated effects were associated mostly with non-polar compounds (sampled by the silicone rubber sampler) the rest of toxic potentials was associated mainly with more polar chemicals sampled by the EMPORE disc samplers. To link the toxic potentials with chemical analysis data, the detected pollutant levels and their relative effect potencies were used for calculation of bioanalytical equivalent concentrations (BEQs) using the concentration addition concept, with effect concentrations obtained from literature or US EPA ToxCast database. BEQs served for explaining the observed bioassay-derived toxic potentials and identification of the main drivers of toxicity. Our work demonstrates the utility of passive sampling for analysis of trace contaminants in river water as well as effect-based monitoring. The SOLUTIONS Project is supported by the Seventh Framework Programme (FP7-ENV-2013) of the European Union under grant agreement no. 603437.

WEPC18
COMPLEMENTARITY OF PASSIVE SAMPLERS AND PASSIVE BIO-MONITORING TO EVALUATE SURFACE WATER CONTAMINATION BY PESTICIDES IN VINEYARD CATCHMENT
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Grab sampling may be scarcely adequate to evaluate the impact on surface waters of pesticide used in agriculture because of its poor spatiotemporal representativeness compared to the high variability of the contamination patterns. Indeed, it is still not easy to link the effect of agricultural practice changes to water quality at the catchment scale. Furthermore, biological indicators may also be useful in addition to pesticide concentration determination to evaluate their impact on ecosystems. In this way, the contribution of new integrative tools has been evaluated. Passive samplers and biological indicators have been applied together at the catchment scale so as to identify their coherence and complementarity. The tested chemical tool is the Silicone Rod (SR) dedicated to hydrophobic to moderately hydrophilic compounds. In total, 22 pesticides (12 herbicides, 5 fungicides and 5 insecticides) were analyzed as well as potential confusing parameters (metal(oïd)s, nutrients, temperature). In parallel the biological approach consisted in an *in situ* study of i) leaf litter decomposition processes, which are mediated by the combined action of microbial and invertebrate communities and of ii) the measurement of fungal biomass within leaf-associated microbial communities. These tools have been applied during June 2014 and June 2015 (corresponding to a period of pesticides application on vineyard) in Ardières river. The Ardières basin, situated in the Beaujolais region (France), is submitted to viticulture pressure and a marked pesticide contamination has already been highlighted. Three measurement stations have been chosen along the contamination gradient from upstream to downstream. Results showed the high coherence of chemical and biological tools responses. All of them were able to reflect the spatial contamination gradients as well as the specific contamination and impact associated. Thus, decomposition rates and fungal biomass decreased from up to downstream, in relation to the observed increase in fungicides and organophosphorus insecticides concentrations. This biological impact was observed during the two years studied, while pesticide contamination decreased in 2015 compared to 2014. Finally, their sensitivity, reactivity and integration capacity make these tools relevant and promising to assess agricultural pesticide impact on surface water in addition to grab sampling.

WEPC19

From in-situ passive sampling into bio assays - an innovative approach of combined chemical and biological analysis of HOCs

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The proposed project aims at the development of innovative indicators to allow for a spatially structured description and assessment of the pollution and the risk potential of sediment-bound contaminants in marine ecosystems. For the first time the project will obtain toxicity data for the pore water concentrations of pollutants with largely reduced uncertainty, directly correlate the results with chemical analysis and verify the findings using artificial mixtures of the target analytes. This will be achieved by adaptation of an in situ equlibrium sampler (passive sampler) based on solid phase microextraction (SPME) for the investigation of hydrophobic organic compounds in marine ecosystems. The PDMS hollow fibers carrying the sampled pollutant mixtures will then be directly applied as passive dosing phases in small-scale biotest systems. As hence no extraction of the fibers is necessary, the risk to alter the original substance composition is significantly reduced. Thus, resulting data are highly representative for the actual pollution on-site. Subsequently, the analysed mixture will be reassembled and tested with concentration series using passive dosing in the test systems to derive concentration-response curves. Together with results on the distribution and accumulation of the contaminants an estimation of the risk due to sediment-bound pollutants will be possible (mixture toxicity). Such realistic toxicity data in combination with knowledge on the distribution of the compounds and correlated with sediment parameters are a good foundation to derive reliable indicators for a good environmental state of Baltic Sea waters.

Keyword Index

Accumulation. 147,198,21,223,279,29,297,299,300,301,302,303,304,305,306,307,308,309,310,311,312,313,314,315,316,317,32,324,330,383,427,454,455,456,457,458,459,470,471,473,474,48,49,50,51,52,53,54,55,MO004,MO033,MO056,MO060,MO079,MO081,MO145,MO148,MO153,MO153,MO164,MO184,MOPC11,TH055,TH064,TH065,TH112,TH144,TU002,TU008,TU042,TU108,WE047,WE063,WE070,WE111,WE120,WE165

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13,176,203,204,205,206,207,254,276,332,333,348,518,MO004,MO105,MO107,MO250,MO276,MO277,TH082,TH111,TH128,TH132,TH132,TU058,TU058,TU083,TU092,TU118,TU211,WE046,WE135,WE163,WE169,WE169

Landscape.
23,364,387,388,MO219,MO251,TU159,TU160,TU161,TU162,TU193,TU193,TUPC01,TUPC02,TUPC06,TUPC14,TUPC15,TUPC16,TUPC17,TUPC18,WE163,WE195,WE195,WE249,WE256

Life-cycle assessment.
103,104,105,106,107,108,156,157,158,159,160,161,208,209,210,211,212,250,258,259,260,261,262,291,292,293,294,295,296,355,364,365,366,367,368,369,401,417,418,419,42,420,421,43,44,45,46,460,461,462,463,464,465,466,467,468,469,47,521,522,523,524,525,572,573,574,575,576,577,92,MO026,MO280,MO281,MO282,MO283,MO284,MO285,MO286,MO287,TH045,TH177,TH178,TH179,TH179,TH181,TH181,TH183,TH184,TH185,TH186,TH187,TH188,TH189,TH190,TH191,TH192,TU049,TU076,TU076,TU205,TU222,TU223,TU224,TU225,TU226,TU227,TU228,TU229,TU230,TU230,TU231,TU231,TU232,TU232,TU233,TU233,TU234,TU235,TU236,TU238,TU239,TU240,TU241,TU242,TU243,TU244,TU245,TU246,TU247,TU248,TU249,TU250,TU251,TU252,TU253,TU254,TU255,TU256,TU257,TU258,TU259,TU260,TU261,TU262,TU262,TU263,TU263,TU264,TU264,TU265,TU265,TU270,WE247,WE248,WE249,WE250,WE251,WE252,WE253,WE254,WE255,WE256,WE257,WE257,WE258,WE258,WE259,WE259,WE260,WE260

Mesocosm.
112,202,204,205,206,213,332,390,433,447,515,MO123,MO135,MO176,TH060,TH118,TH122,TH123,TH126,TU021,TU021,TU023,TU023,TU061,TU061,TU209,TU211,TU267,TU269

Metabolism.
142,170,21,231,284,285,3,30,354,409,446,527,529,531,539,540,58,MO097,MO135,TH018,TH070,TH115,TH125,TH137,TU011,TU074,TU074,TU087,TU090,TU153,TU191,TU259,WE060,WE066,WE139,WE172,WE177,WE182,WE183,WE193,WE193

Metalloids.
141,480,80,MO137,TU151,TU174,TU199,TU203

Metals.
136,138,139,142,143,167,191,192,193,194,195,201,237,24,246,247,248,249,25,250,251,26,27,277,28,29,290,293,324,325,331,377,382,403,430,431,435,439,446,482,483,484,485,505,533,541,542,80,81,82,83,84,85,MO080,MO093,MO097,MO104,MO116,MO124,MO126,MO131,MO135,MO136,MO137,MO138,MO157,MO158,MO159,MO160,MO161,MO162,MO163,MO164,MO167,MO170,MO171,MO172,MO173,MO174,MO175,MO176,MO177,MO179,MO180,MO181,MO182,MO183,MO185,MO186,MO262,MO274,TH029,TH030,TH032,TH033,TH034,TH035,TH036,TH037,TH038,TH040,TH041,TH042,TH043,TH044,TH046,TH049,TH050,TH05

3,TH054,TH058,TH090,TH093,TH099,TH101,TH104,TH116,TH125,TH130,TH130,TH147,TH164,TH167,TH175,TH175,TU005,TU046,TU059,TU059,TU061,TU061,TU096,TU097,TU098,TU099,TU100,TU101,TU102,TU104,TU106,TU107,TU109,TU110,TU111,TU112,TU114,TU117,TU132,TU135,TU152,TU153,TU157,TU157,TU176,TU181,TU192,TU192,TU195,TU197,TU198,TU200,TU201,TU204,TU205,TU239,WE012,WE015,WE016,WE017,WE021,WE026,WE048,WE049,WE077,WE107,WE108,WE116,WE117,WE131,WE132,WE171,WE171,WE224,WE226,WE228,WE229,WEPC07

Microcosm.
140,174,203,207,218,428,445,83,91,MO034,MO041,MO047,MO088,MO088,MO189,TH008,TH042,TH080,TH086,TH120,TH122,TU001,TU092,TU097,TU099,TU100,TU209,WE084,WE196,WE196,WE204

Mixture toxicity.
1,126,167,176,181,192,193,194,196,213,219,229,240,241,243,244,245,287,288,289,290,348,357,409,431,440,441,442,502,503,553,558,78,98,MO017,MO080,MO084,MO092,MO108,MO142,MO195,MO196,MO208,TH024,TH025,TH067,TH118,TH119,TH127,TH132,TH132,TU014,TU017,TU018,TU021,TU021,TU022,TU022,TU079,TU084,TU096,TU097,TU103,TU104,TU107,TU109,TU110,TU114,TU120,TU135,TU137,TU152,TU157,TU157,TU158,TU158,TU179,TU181,TU183,TU184,TU185,TU186,TU187,TU188,TU190,TU192,TU192,TU193,TU193,TU194,TU194,TU196,WE066,WE094,WE123,WE133,WE185,WE186,WE194,WE194,WE207,WE222,WE234,WEPC17,WEPC19

Monitoring.
1,101,114,12,131,16,162,166,186,235,248,265,288,325,326,327,328,353,371,375,381,425,480,484,494,505,507,511,515,518,555,557,560,563,57,63,64,65,68,69,7,70,71,72,84,99,MO004,MO009,MO014,MO016,MO028,MO030,MO033,MO035,MO037,MO039,MO043,MO051,MO053,MO057,MO060,MO061,MO062,MO063,MO067,MO069,MO183,MO191,MO205,MO222,MO224,MO229,MO229,MO237,MO238,MO246,MO263,MO279,MOPC01,MOPC08,TH033,TH040,TH043,TH079,TH119,TH133,TH135,TH137,TH143,TH145,TH151,TH166,TH166,TH168,TH170,TH173,TU006,TU007,TU009,TU012,TU062,TU063,TU066,TU073,TU077,TU077,TU080,TU105,TU141,TU168,TU169,TU185,TU189,TU198,TU268,TU273,TUPC11,WE034,WE035,WE046,WE051,WE051,WE056,WE058,WE069,WE070,WE071,WE074,WE076,WE077,WE081,WE081,WE104,WE104,WE106,WE106,WE112,WE141,WE141,WE178,WE222,WE240,WE247,WEPC07,WEPC15

Multimedia.
36,373,38,39,419,558,568,95,MO009,MO011,MO052,MO067,MO147,MO175,MO216,MO217,MO220,MO221,MO223,MO224,MO225,MO226,MO226,MO229,MO229,MO237,TH146,WE072,WE189,WEPC05,WEPC06

Mutagenicity.
509,TH139,TH143,WE118,WEPC15

Nanomaterials.
115,116,117,118,119,120,140,173,174,175,176,177,178,180,184,215,216,219,220,221,222,223,239,269,270,271,272,273,323,324,325,326,327,328,355,36,37,376,377,378,379,380,381,433,434,435,436,437,448,486,487,489,490,491,493,532,533,534,535,536,537,546,550,MO042,MO082,MO158,MO212,MO217,MO249,TH015,TH044,TH045,TH046,TH048,TH049,TH050,TH051,TH052,TH053,TH054,TH055,TH056,TH057,TH058,TH059,TH060,TH061,TH062,TH063,TH064,TH073,TH162,TU024,TU026,TU027,TU028,TU029,TU030,TU030,TU031,TU031,TU032,TU032,TU033,TU033,TU034,TU035,TU036,TU038,TU039,TU040,TU041,TU042,TU043,TU044,TU045,TU046,TU047,TU048,TU049,TU052,TU053,TU054,TU055,TU057,TU058,TU058,TU059,TU059,TU060,TU060,TU061,TU061,TU271,WE008,WE009,WE024,WE099,WE099,WE100,WE101,WE102,WE104,WE104,WE105,WE105,WE191,WE192

Natural resource damage.
368,392,498,571,76,TH104,WE254

Nutrients.
117,218,544,MO007,TH132,TH132,TU260,WE004,WE067,WE137,WE138,WE210,WE258,WE258

Partitioning.
121,127,158,225,335,357,37,372,373,38,393,394,395,526,561,6,62,MO001,MO011,MO054,MO072,MO074,MO147,MO216,MO229,MO229,MO232,MOPC10,MOPC11,TH188,TU171,WE010,WE019,WE034,WE035,WE038,WE039,WE040,WE041,WE042,WE072,WE150,WE173

Passive sampling.
187,298,338,370,371,372,373,375,378,422,423,424,425,426,497,514,64,78,MO052,MO121,TH043,TH081,TU010,TU011,TU065,WE025,WE027,WE028,WE029,WE029,WE030,WE031,WE032,WE033,WE034,WE035,WE036,WE038,WE039,WE040,WE041,WE042,WE043,WE044,WE045,WE046,WE047,WE048,WE049,WE050,WE051,WE051,WE052,WE052,WE053,WE053,WE058,WE059,WE064,WE221,WEPC14,WEPC15,WEPC17,WEPC18,WEPC19

Persistent.
122,128,163,185,186,189,190,227,263,264,265,266,337,370,374,384,40,475,476,477,478,538,562,564,565,566,62,64,65,66,67,90,93,99,MO011,MO051,MO053,MO059,MO060,MO061,MO062,MO064,MO065,MO074,MO083,MO147,MO227,MO227,TH005,TH007,TH009,TH011,TH011,TH016,TH028,TH065,TH066,TH073,TH076,TH095,TH173,TU006,TU067,TU068,TU070,TU071,TU074,TU074,TU077,TU077,TU138,TU173,WE006,WE019,WE055,WE059,WE060,WE063,WE064,WE074,WE077,WE078,WE078,WE109,WE120,WE124,WE126,WE198,WE221,WE243

Personal care product.
102,109,110,114,2,353,354,355,356,38,445,505,564,92,94,95,MO027,MO033,MO036,MO037,MO049,MO090,MO090,MO120,MO141,MO146,MO211,MO223,MO224,TU049,TU051,TU093,TU165,WE005,WE074,WE125,WE154,WE196,WE196,WE197,WE198,WE199,WE200,WE201,WE202,WE204,WE206,WE208,WE209,WE214

Pesticide.
101,144,145,146,148,149,162,163,166,19,199,200,213,243,253,255,256,257,258,259,281,288,319,385,410,412,417,419,493,501,527,541,548,60,79,96,MO001,MO004,MO030,MO114,MO115,MO118,MO129,MO133,MO144,MO149,MO150,MO154,MO154,MO155,MO155,MO156,MO156,MO208,MO219,MO222,MO230,MO231,MO232,MO233,MO234,MO235,MO236,MO238,MO241,MO242,MO243,MO244,MO245,MO247,MO251,MO254,MO255,MO257,MO258,MO260,MO263,MO264,MO267,MO278,MOPC01,TH008,TH014,TH017,TH066,TH070,TH082,TH089,TH091,TH120,TH126,TH127,TH128,TH129,TH129,TH131,TH131,TH141,TH144,TH148,TH154,TH156,TH167,TH176,TH176,TU007,TU018,TU120,TU121,TU134,TU142,TU144,TU160,TU162,TU187,TU193,TU193,TU208,TU265,TU265,TU268,TU273,TUPC01,TUPC02,TUPC03,TUPC07,TUPC15,TUPC16,TUPC18,TUPC19,WE001,WE007,WE018,WE028,WE029,WE029,WE030,WE031,WE037,WE044,WE052,WE052,WE053,WE053,WE059,WE060,WE066,WE081,WE081,WE101,WE114,WE145,WE145,WE146,WE146,WE162,WE164,WE165,WE172,WE185,WE186,WE220,WE221,WE225,WE227,WE256,WEPC18

Pharmaceuticals.
134,15,165,168,169,170,171,172,196,197,198,21,216,281,287,3,319,322,350,4,406,411,413,414,415,416,424,425,428,429,430,432,438,443,5,507,527,547,555,56,561,564,59,6,60,61,93,MO008,MO010,MO013,MO014,MO016,MO021,MO026,MO035,MO036,MO039,MO044,MO045,MO091,MO099,MO100,MO101,MO113,MO146,MO213,MOPC07,TH007,TH020,TH145,TU001,TU015,TU016,TU017,TU020,TU020,TU021,TU021,TU023,TU023,TU056,TU115,TU145,TU164,TU165,TU269,TUPC14,WE002,WE003,WE023,WE032,WE043,WE045,WE082,WE083,WE084,WE085,WE095,WE096,WE097,WE098,WE125,WE128,WE142,WE142,WE181,WE211,WE215,WE219,WE231,WE232,WE233,WE234,WE235,WE236,WE237,WE238,WE239,WE240,WE241,WE242,WE243,WE244,WE245,WE246,WE247,WE248

Policy analysis.
369,398,549,560,57,570,571,573,TH030,TH088,TH107,TH108,TH109,TH149,TH152,TH174,TH182,TH182,TU163,TU225,TU235,TU238,TU244,TU252,TU270,TU272,WE199,WE225,WE252,WEPC01,WEPC02

Regulation.
145,151,186,227,246,269,271,282,284,346,352,360,363,485,504,510,556,562,565,566,567,75,MO019,MO043,MO049,MO070,MO156,MO156,MO157,MO247,MO264,MO266,MO269,MO271,MO277,MO288,TH006,TH008,TH030,TH063,TH149,TH154,TH157,TH158,TH159,TH161,TH166,TH166,TH173,TH175,TH175,TH176,TH176,TH180,TH180,TU024,TU025,TU027,TU028,TU030,TU030,TU032,TU032,TU084,TU086,TU093,TU094,TU094,TU106,TU176,TU179,TU180,TU190,TU194,TU194,TU201,TU220,TU232,TU232,TU272,TUPC05,WE030,WE055,WE078,WE078,WE105,WE105,WE136,WE167,WE174,WE190,WE199,WE200,WE202,WE213,WE225,WE239,WE241,WE242,WE246,WEPC02

Remediation.
111,113,218,544,546,90,TH084,TH090,TH096,TH097,TU075,TU075,TU204,TU218,WE008,WE009,WE010,WE012,WE014,WE015,WE022,WE023,WE025,WE026,WE049

Reproduction.
135,14,154,278,331,382,402,451,489,74,75,MO038,MO066,MO068,MO108,MO117,MO128,TH039,TH071,TH072,TH129,TH129,TU003,TU088,TU126,TU212,WE145,WE145,WE193,WE193,WE218,WE219,WE220,WE228

Risk assessment.
1,101,102,124,13,131,132,141,144,145,146,150,151,152,153,155,166,167,185,192,202,204,217,22,241,243,249,25,252,256,257,268,269,270,289,290,298,30,327,328,333,334,34,341,342,343,345,346,351,356,357,358,359,36,362,363,37,377,380,387,394,398,41,416,418,432,436,451,453,475,476,478,504,508,512,513,516,517,518,52

0,536,545,548,555,556,557,563,568,570,571,63,72,74,8,83,87,88,92,96,97,MO013,MO026,MO035,MO046,MO085,MO105,MO106,MO108,MO114,MO143,MO144,MO145,MO155,MO155,MO156,MO156,MO162,MO165,MO167,MO172,MO175,MO177,MO182,MO187,MO202,MO209,MO210,MO214,MO220,MO221,MO226,MO226,MO230,MO231,MO233,MO234,MO239,MO240,MO242,MO244,MO245,MO247,MO250,MO252,MO253,MO258,MO260,MO264,MO265,MO270,MO271,MOPC04,MOPC07,TH005,TH010,TH010,TH031,TH033,TH045,TH047,TH067,TH083,TH085,TH096,TH097,TH111,TH125,TH135,TH141,TH146,TH151,TH153,TH154,TH155,TH156,TH159,TH162,TH163,TH166,TH166,TH169,TH171,TH174,TH176,TH176,TH189,TU010,TU012,TU027,TU030,TU030,TU043,TU050,TU055,TU057,TU064,TU066,TU074,TU074,TU082,TU083,TU092,TU097,TU098,TU107,TU118,TU129,TU149,TU159,TU160,TU161,TU163,TU164,TU169,TU175,TU176,TU178,TU180,TU181,TU182,TU183,TU184,TU185,TU187,TU190,TU191,TU197,TU199,TU200,TU201,TU202,TU203,TU206,TU207,TU208,TU212,TU214,TU216,TU219,TUPC01,TUPC09,TUPC10,TUPC14,TUPC15,TUPC16,WE001,WE007,WE014,WE020,WE072,WE085,WE098,WE110,WE119,WE140,WE140,WE154,WE157,WE162,WE163,WE167,WE168,WE168,WE176,WE190,WE197,WE199,WE202,WE203,WE205,WE213,WE223,WE224,WE235,WE237,WE238,WE239,WE240,WE241,WE242,WE246,WE248,WE251,WEPC03,WEPC08,WEPC14,WEPC16

Risk management.
185,207,251,352,360,411,416,476,504,561,566,569,570,576,63,99,MO005,MO009,MO132,MO187,MO202,MO218,MO238,MO245,MO265,MO272,MO277,MO278,MO279,TH119,TH147,TH152,TH163,TH171,TH189,TU154,TU160,TU163,TU176,TU185,TU218,TU220,TU221,TU232,TU232,TUPC01,TUPC02,TUPC03,TUPC04,TUPC05,WE018,WE238,WE242,WEPC02

Roadway.
208,212,MO185,TU222,TU223,TU228

Sediment.
100,147,181,224,248,253,254,356,357,363,372,373,374,444,447,477,478,490,492,514,574,6,71,80,85,87,88,89,90,91,MO001,MO010,MO020,MO174,MO176,MO177,MO178,MO179,MO185,MO187,MO188,MO189,MO190,MO191,MO218,MO223,MO241,MO256,TH002,TH020,TH047,TH057,TH065,TH098,TH100,TH134,TH140,TH141,TH168,TU015,TU045,TU046,TU062,TU063,TU064,TU070,TU102,TU105,TU122,TU145,TU167,TU199,TU210,TU221,WE022,WE049,WE063,WE073,WE074,WE075,WE076,WE077,WE097,WE119,WE132,WE165,WE169,WE169,WE201,WE204,WEPC19

Soil.
144,146,148,150,151,152,153,154,155,183,214,220,221,27,272,28,327,35,389,415,429,430,446,477,512,528,529,532,533,534,545,547,549,82,83,MO029,MO034,MO041,MO047,MO126,MO131,MO161,MO165,MO167,MO169,MO172,MO220,MO221,MO226,MO226,MO233,MO239,MO240,MO241,MO247,MO248,MO250,MO252,MO253,MO254,MO255,MO256,MO257,MO259,MO263,MO264,MO266,MO269,MO270,TH002,TH004,TH009,TH010,TH010,TH015,TH016,TH017,TH021,TH027,TH037,TH046,TH050,TH053,TH079,TH080,TH083,TH086,TH089,TH091,TH101,TH164,TH165,TH165,TH168,TH170,TH173,TU001,TU026,TU043,TU048,TU052,TU058,TU058,TU061,TU061,TU075,TU075,TU098,TU167,TU175,TU196,TU200,TU204,TU205,TU214,TU258,TU261,TU271,WE009,WE012,WE015,WE016,WE017,WE018,WE020,WE026,WE033,WE034,WE092,WE093,WE094,WE095,WE171,WE171,WE192,WE195,WE195,WE253

Sorption.
100,121,127,129,130,149,177,178,224,264,28,338,393,395,547,59,71,MO020,MO045,MO079,MO147,MO183,MO216,MOPC02,MOPC03,MOPC10,TH016,TH026,TU075,TU075,WE008,WE010,WE013,WE014,WE021,WE032,WE052,WE052,WE095,WE151

Spatial.
186,190,284,345,364,369,413,47,496,5,63,64,69,71,94,MO052,MO055,MO174,MO185,MO211,MO224,MO225,MO226,MO226,MO228,MO228,MO279,TU071,TU250,TU253,TU263,TU263,TUPC06,TUPC19,WE061,WE076,WE159,WE209,WE258,WE258,WE259,WE259

Speciation.
191,223,24,246,247,25,250,26,27,29,377,378,435,480,485,MO031,MO157,MO158,MO159,MO160,MO161,MO168,TH029,TH038,TH042,TH075,TH167,TU066,TU195,TU196,WE047,WE050

Statistics.
137,239,336,339,342,347,369,516,537,85,MO150,MO153,MO153,MO252,MO267,MO281,TH064,TH155,TH156,TH160,TU005,TU024,TU030,TU030,TU055,TU087,TU126,TU165,TU192,TU192,TU209,TU251,WE056,WE075,WE167,WE168,WE168,WE182,WE201

Stormwater.
100,161,318,4,MO058,MO275,MOPC01,TH122,WE042

Surface water.
101,146,147,16,166,191,2,224,240,241,242,243,265,267,268,283,289,318,319,320,321,34,376,381,408,413,434,437,443,47,496,506,508,509,518,526,528,544,574,58,68,69,70,80,93,94,95,96,MO003,MO010,MO015,MO016,MO023,MO027,MO044,MO058,MO069,MO076,MO087,MO087,MO090,MO090,MO125,MO138,MO194,MO195,MO212,MO219,MO228,MO228,MO229,MO229,MO233,MO235,MO243,MO244,MO288,TH025,TH043,TH045,TH060,TH098,TH103,TH123,TH153,TU004,TU011,TU013,TU031,TU031,TU044,TU055,TU062,TU065,TU118,TU199,TU268,TU269,TUPC10,TUPC16,WE028,WE040,WE042,WE043,WE044,WE046,WE052,WE052,WE059,WE063,WE067,WE068,WE069,WE070,WE071,WE073,WE090,WE096,WE097,WE101,WE104,WE104,WE106,WE106,WE114,WE162,WE209,WE210,WEPC17,WEPC18

Sustainability.
102,105,160,208,210,212,262,292,293,294,295,421,43,44,472,498,517,521,522,524,525,572,575,576,577,MO025,MO065,MO207,MO280,MO281,MO284,MO285,TH081,TH088,TH107,TH108,TH109,TH177,TH178,TH180,TH180,TH181,TH181,TH182,TH182,TH184,TH187,TU224,TU228,TU233,TU233,TU235,TU241,TU243,TU249,TU250,TU255,TU256,TU257,TU258,TU262,TU262,TU263,TU263,TU270,TUPC04,TUPC06,WE243,WE249,WE252,WE253

Systems analysis.
105,106,115,165,210,261,351,386,389,391,399,447,499,521,525,571,572,8,MO215,MO284,MO286,TH088,TH107,TH108,TH109,TH169,TH175,TH175,TH177,TH181,TH181,TH182,TH182,TH191,TU066,TU226,TU232,TU232,TU235,TU237,TU239,TU241,TU252,TU257,TU258,WE175,WE184,WE252

Toxicity.
115,118,128,132,138,143,164,196,223,228,253,298,30,320,33,334,343,344,347,349,382,385,394,398,418,419,431,487,511,513,523,532,536,562,565,87,88,MO002,MO024,MO042,MO065,MO068,MO083,MO089,MO089,MO141,MO148,MO157,MO163,MO167,MO172,MO173,MO181,MO188,MO197,MO202,MO203,MO204,MO231,MO256,MO268,MO274,MOPC13,TH006,TH010,TH010,TH034,TH035,TH048,TH052,TH077,TH081,TH089,TH094,TH100,TH145,TH148,TH162,TH180,TH180,TU002,TU019,TU033,TU033,TU038,TU039,TU048,TU059,TU059,TU078,TU080,TU082,TU085,TU111,TU114,TU128,TU135,TU143,TU144,TU175,TU182,TU205,WE002,WE078,WE078,WE099,WE099,WE125,WE126,WE132,WE134,WE135,WE136,WE152,WE167,WE175,WE184,WE203,WE227,WE228,WE248,WEPC16

Toxicokinetics.
10,120,139,22,226,230,275,277,285,342,343,344,396,403,439,481,77,MO170,MO171,TH057,TH058,TH068,TU018,TU056,TU101,TU159,TU161,TU162,TU172,WE027,WE110,WE156,WE157,WE158,WE161,WE169,WE169,WE172

Uncertainty.
103,104,106,160,252,287,289,345,358,36,395,40,558,94,MO153,MO153,MO211,MO230,MO283,MO288,TH155,TH158,TH174,TU141,TU159,TU161,TU206,TU226,TU265,TU265,WE150,WE152,WE190,WE205,WEPC02

Urban.
161,318,331,379,544,68,73,MO009,MO011,MO036,MO053,MO058,MO135,MO212,MO240,MO270,MO275,MOPC02,MOPC05,MOPC06,TH033,TH101,TU002,TU233,TU233,TU238,WE108,WE136,WEPC01

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